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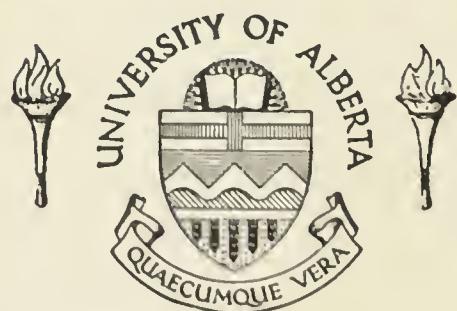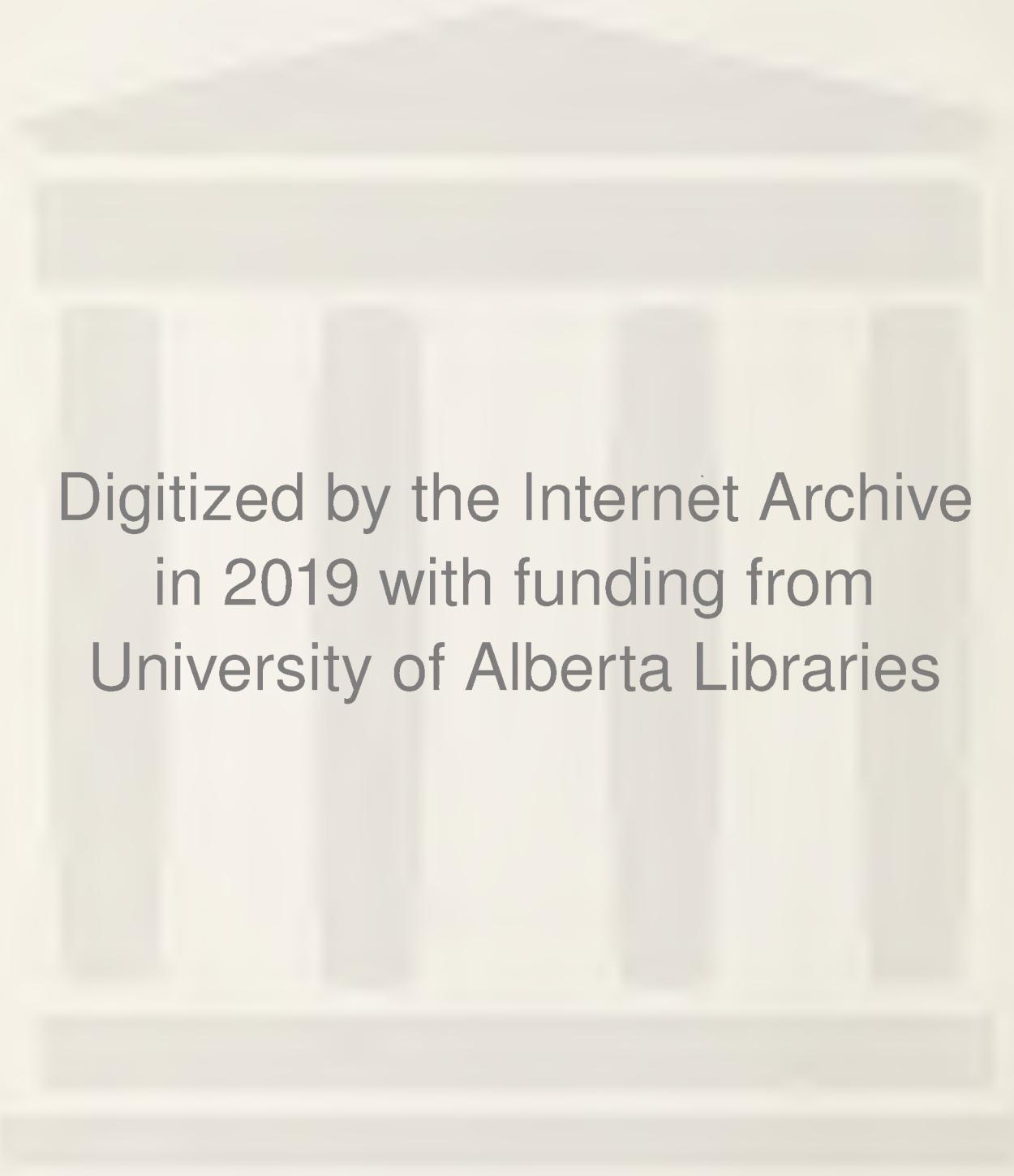
UNIVERSITY OF ALBERTA

THE MECHANISM OF AN EXPERIMENTAL UNILATERAL OLIGURIA

A THESIS

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ABSTRACT

An investigation of the unilateral oliguria produced by installation of a bubble flowmeter into the left renal vein was undertaken. Previous investigations showed that this maneuver produced severe unilateral oliguria associated with a decrease in GFR, a low extraction ratio of PAH, a low filtration fraction, with a normal total RBF as measured with the flowmeter and calculated by PAH extraction. This was taken to be indicative of an intrarenal diversion of blood from the highly functional renal cortex to the less functional renal medulla, possibly through the juxtamedullary glomeruli.

Preliminary investigations indicated that the extraction ratio of PAH was normal and that the low values reported in the previous study could have been caused by failure to separate the red blood cells from the plasma soon enough after sampling to prevent passage of PAH from the red blood cells into the plasma in the venous samples. It was also shown by intra-arterial injections of India ink after meter installation that the blood flow was not shunted from the cortex to the medulla. On the contrary, there was a slight indication that the reverse occurred.

The experimental data of these preliminary investigations showed that the oliguria could be accounted for in most cases by a unilateral decrease in GFR. There was some indication that it was sometimes due to unilateral augmentation of the concentrating mechanism. There was no obvious cause for these findings but it was suspected that the meter installation may have caused a larger increase in renal venous

pressure than had been previously reported.

An investigation on ten dogs, all of which were made to produce concentrated urine by administration of ADH, and in which changes in renal venous pressure were measured by a more accurate method than had formerly been used showed that meter installation produced moderate unilateral oliguria in most cases. A substantial increase in renal venous pressure was implicated as the etiological factor since further increases caused more pronounced oliguria seven out of ten times. The mechanism of the increased pressure appeared to be both mechanical and reflex in nature since infiltration of anaesthetic in the peripelvic region, modified the response but did not abolish it or prevent it.

The ultimate factors producing changes in urine production were found to be the glomerular filtration rate and the urine concentrating mechanism working separately or together. A hypothesis involving the juxtamedullary circulation and the countercurrent theory of urine concentration is offered as an explanation of the results obtained.

It was decided in view of the fundamental changes in renal function produced by the installation of the meter into the renal vein, that meters which raise the renal venous pressure should not be used to measure changes in total renal blood flow.

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CHAPTER ONE

INTRODUCTION

Note:- Conventional and non-conventional abbreviations
have been used throughout this thesis. A guide
to these may be found in Appendix I.

The role of the kidney in controlling blood volume has not yet been fully elucidated. Investigations by Young et al. (1955) on dogs showed that although a diuresis occurred after the infusion of various plasma expanders, the glomerular filtration rate (clearance of creatinine) did not increase and the effective renal blood flow (clearance of PAH) remained normal or fell. It was therefore inferred that at least part of the diuresis was due to alteration in tubular function mediated through a decrease in blood levels of ADH. This group suggested that the mechanism of the decrease could be due to a cessation of release of the hormone from the neuro-hypophysis in response to stimulation of "volume" receptors in the thoracic viscera. The work of Zuidema et al. (1956) supported these findings. An investigation by Atkins and Pearce (1959) showed that expansion of plasma volume in dogs by use of plasma or iso-oncotic albumin solution resulted in a diuresis associated with an increase in ERBF and no consistent change in GFR. This response was reduced by cervical vagotomy which appeared to indicate that "volume" receptors other than in the thoracic viscera may contribute to the reflex nature of the volume-control diuretic mechanism.

Effective RBF, used by the above mentioned investigators, is an adequate estimate of renal plasma flow, provided that the extraction ratio of PAH, the compound used for this determination, does not change as a result of the volume expansion. Determination of RBF by this method also has the

disadvantage characteristic of all clearance determinations, in that rapid changes in function cannot be detected, because of the necessity to collect urine over a timed interval. Only the average flow rate during this interval can be calculated. It was thought that further information regarding the possible hemodynamic renal mechanics of blood volume control could be obtained by using a direct method for determining the change in renal blood flow rather than the indirect clearance method.

Direct methods of measuring RBF were pioneered by Selkurt (1945) who used a bubble flowmeter to measure renal venous outflow. Owing to the unique blood supply and drainage of the kidney, this measurement is equivalent to the total renal blood flow. Selkurt found that TRBF measured simultaneously by extraction rather than clearance of PAH, was the same as the meter reading, and that the installation of the meter had no apparent effect upon the glomerular filtration rate (GFR) or urine production (V). These findings were confirmed by Conn and Markley (1950) and by Study and Shipley (1950) using different types of flowmeters.

Renal venous outflow was measured with a bubble flowmeter in this laboratory in an attempt to determine the effect of intravascular volume expansion on the total RBF. Selkurt's procedures (1945) were modified in that very little intravenous fluid was administered to the experimental animal before the actual volume expansion. However under the conditions

of these experiments, it was found that the experimental or
left kidney almost always produced considerably less urine
that the control or right kidney, although the left renal
blood flows measured with the flowmeter agreed well with other
published normal values. Because it was felt that this
observed unilateral oliguria probably indicated some change
in renal function which might affect the response to hyper-
volemia, it was decided to investigate the mechanism of the
phenomenon before continuing with the hypervolemia studies.

The observation that the blood flow rates recorded
by the meter were close to the normal level, in spite of
reduced urine production in that kidney, suggested that an
intrarenal shunting of blood from functional to non-functional
tissue could have been occurring. Trueta et al. (1947)
postulated such a shunt through the juxtamedullary circulation
of the rabbit and believed they were able to demonstrate it.
It was also possible that an increase in renal venous pressure
could have reduced the GFR by increasing pressure within the
kidney, although moderate increases do not affect renal function
to any extent (Selkurt et al. 1949, Blake et al. 1949). A
third possibility was interference with the concentrating
mechanism of the kidney causing more water to be reabsorbed
and thus decreasing urine volume. Any of these factors
individually or in combination with the others could have
produced the unilateral oliguria.

On the basis of experiments performed on 25 dogs,

Van Petten (1959) concluded that the observed unilateral
oliguria was due to intrarenal shunting of blood. Van Petten
combined functional tests with the meter installation and
reported that placing a bubble flowmeter into the left renal
vein of dogs produced a decrease in GFR(creatinine clearance),
a decrease in true filtration fraction (creatinine clearance
/TRPF), a decrease in E_{PAH}, and a decrease in urine production
in the left kidney, with little or no decrease in the total
blood flow as measured by E_{PAH} or the meter. These conditions
would indicate that a shunting of blood from functional to
less or non-functional tissue had occurred (Robinson 1954 p.43).
Van Petten also found that administration of an osmotic diuretic,
mannitol, did not change these relationships and that if the
kidneys were pharmacologically denervated by infiltration with
a local anaesthetic during a mannitol diuresis, there was
considerably less effect produced by the installation of the
meter. He concluded that the oliguria was caused by a cortex
to medulla shunting of blood, related to the shunting of blood
in rabbit kidneys described by Trueta et al. (1947). He
suggested that this was initiated reflexly by introduction of
the flowmeter pickup catheter into the renal vein, similar
to "veno-renal" reflex described by Friedman et al (1956),
and that the failure of local anaesthetic to correct the
condition completely was due to incomplete blocking.

Examination of Van Petten's protocol books showed
that he had failed to take the precautions suggested by

Phillips (1945) to prevent passage of PAH from the red blood cells to the plasma in the renal venous samples used to determine E_{PAH}. Preliminary experiments (discussed in Results part I and part II) showed that this rate of passage was sufficiently rapid to be responsible for the low extraction ratios he reported. In addition perfusion studies of the kidneys with India ink, after installation of the meter, failed to demonstrate intrarenal diversion of blood from the cortex to the medulla. On the basis of these tests the "shunt" hypothesis was rejected.

Reduction of GFR, therefore, was obviously a major cause of the unilateral oliguria, since Van Petten reported large decreasesin GFR in every case. The mechanism producing this decrease was not obvious, since he reported adequate arterial pressures of above 80 mm Hg (Shipley and Study 1951) and low renal venous pressures of less than 10 mm Hg which would not interfere with GFR (Selkurt et al. 1949, Blake et al 1949). However, because he measured venous pressures from a side arm in the flow circuit, instead of from the tip of a catheter in the renal vein, the pressures he measured were between the actual renal venous pressure and intrathoracic pressure (the site of outflow from the meter) and were lower than the actual venous pressures.

Examination of data from the preliminary investigations in this report showed that changes in the concentrating mechanism might also be involved in the production of the oliguria.

Table I - L/R Uosm and V Ratios

Dog	Before Meter			After Meter	
	V ratio	Uosm		V ratio	Uosm
8/18/59	1.07	0.91		0.98	0.84
8/25/59*	1.04	1.04		0.41	1.01
9/8/59	1.06	0.98		0.81	0.97
9/9/59	0.67	1.12		0.39	1.26
9/10/59	1.00	1.20		0.86	1.01
9/11/59	1.00	1.04		0.92	0.99
10/15/59	0.91	1.49		0.57	1.36
10/17/59	0.94	1.18		0.66	1.14
10/22/59	0.99	1.05		0.73	1.28

* R/L ratio, the right renal vein was canulated.
Absolute values are found in Appendix II.

Table I shows that out of nine cases where the left over
right urine volume ratios decreased after meter installation
the left over right urine osmolality ratios increased twice.
It was thought that the incidence of involvement of the con-
centrating mechanism might increase if meter installation
was performed on kidneys which were concentrating maximally
and that more information about the role of the concentrating
mechanism in this unilateral oliguria might be obtained. It
was decided therefore to continue the study on maximally con-
centrating kidneys by using ADH. Since there was a possibility
of error in the previous measurements of the renal venous
pressure increase after meter installation a more accurate
measurement of this parameter was devised. Details of the
procedures used may be found in the "methods" section.

CHAPTER TWO

LITERATURE REVIEW

1.   Introduction

A survey of the literature was undertaken to correlate the findings of previous workers. These findings were used to formulate the experimental plan. The main references used were; "The Kidney" by Homer Smith (1951), "Reflections on Renal Function" by Robinson (1954) and a review article, "The Renal Medulla, Structure, Metabolism and Function" by Ullrich (1960).

2.   Renal Anatomy

(a) Zone Classification (Ullrich 1960)

The kidney can be macroscopically devided into striations or zones because similar histological structures occur at the same levels. These are, from the outside inwards, a brown section known as the cortex, a reddish section known as the outer medulla and a white section known as the inner medulla. On the basis of histological structure, which is discussed below, the outer medulla is often divided into an inner and outer strip.

(b) The Nephron Unit

In the following description the gross anatomy is taken from Sperber (1944), and the histology is from Pease (1955) and Rhodin (1958). The nephron unit is the basic functional unit of the kidney. Its components are distributed within the kidney substance in a typical fashion and are responsible for the aforementioned "zones". The structure relationships are indicated in Figure I. (page 10a)

Each normal nephron unit begins with a glomerulus, a tuft of capillaries enclosed by a double membrane of epithelial tissues. The cells adjacent to the capillaries are thin and interdigitate with each other. Those on the exterior are more cuboidal. The glomerulus drains into a tortuous tubule composed of cuboidal cells containing many mitochondria and possessing a brush border on the inside. This is known as the proximal convoluted tubue (PCT). Both these structures occur only in the cortex.

The PCT straightens out and descends towards the medulla forming the pars recta. The pars recta becomes thin soon after it enters the outer medulla and this point delimits the junction between the inner and outer strip. From there down into the inner medulla the tubule is known as the thin descending loop of Henle. Somewhere in the inner medulla the tubule doubles back and ascends towards the cortex. At the junction between the inner and outer medulla it becomes thick and is known as the thick ascending loop of Henle. The cells of the thin loop are flat and contain few mitochondria. Their nuclei bulge into the cell lumen and the cells interdigitate. Those in the thick portion contain many mitochondria grouped at the base. The bases of the cells are interfolded considerably increasing their total area.

The tubule continues upward almost to the glomerulus and then becomes tortuous again forming the distal convoluted tubule (DCT).

The straight portion in the cortex is known as the intercalated segment and is composed of clear epithelium. The DCT is similar in structure to the thick portion of the loop.

Several tubules join together to form a collecting duct. Typical duct cells are clear cuboidal epithelium with moderate interfolding at the base. Distributed among these cells in ever decreasing numbers towards the papilla are so called 'spurious' cells. These contain many mitochondria and have a large base extending under adjacent cells with much interfolding present. The collecting ducts run an almost straight course through the cortex and outer medulla to the inner medulla where they join together to form larger ducts which drain into the renal pelvis.

The above description is essentially correct for most mammalian kidneys. However, Sperber (1944) found that many mammals have short "cortical nephrons", nephron units which turn back in the outer medulla, or inner cortex, and have only a short thin segment (See Fig. 1). This type is more common in hervivores and some (baboon, beaver, elephant) have kidneys containing only "cortical nephrons" and no inner medullary zone at all. Conversely, some carnivores like the cat and dog have no "cortical nephrons".

(c) Blood Distribution

No extensive studies of the pattern of the distribution of blood vessels in the kidney, which compares with Sperber's

work on the tubule, has been done, but available evidence
indicates the distribution is similar in most mammals. Most
of the following description is taken from Trueta et al. (1947).
The description of the juxtamedullary circulation is from
Lovatt-Evans (1956 p 1015).

Blood is supplied to the kidney by the renal artery,
a single vessel in most mammals which springs directly from
the abdominal aorta. The artery splits before it reaches the
kidney and enters the renal substance at the approximate level
of the outer strip. At this point the arcuate arteries are
formed, which curve across the interior of the kidney in the
outer strip. Stout branches extend radially from the arcuate
arteries to form the interlobular arteries, from which short
afferent arteries lead to the glomeruli, where filtration occurs.
An efferent arteriole drains the glomerulus and splits up
and joins with branches from other efferent arterioles to
form the peritubular capillary network which surrounds the
distal and proximal convoluted tubules. The peritubular cap-
illaries rejoin to form interlobular veins which in turn form
the arcuate veins. A significant number of capillaries run
directly into the arcuate veins.

This scheme is general except for the glomeruli just
adjacent to the medulla, the juxtamedullary glomeruli. The
main outflow from these glomeruli does not flow into the peri-
tubular network (See Fig. 1). The efferent arterioles dip
down into the medulla to a variable depth and then double back

Figure 1 - Renal Anatomy
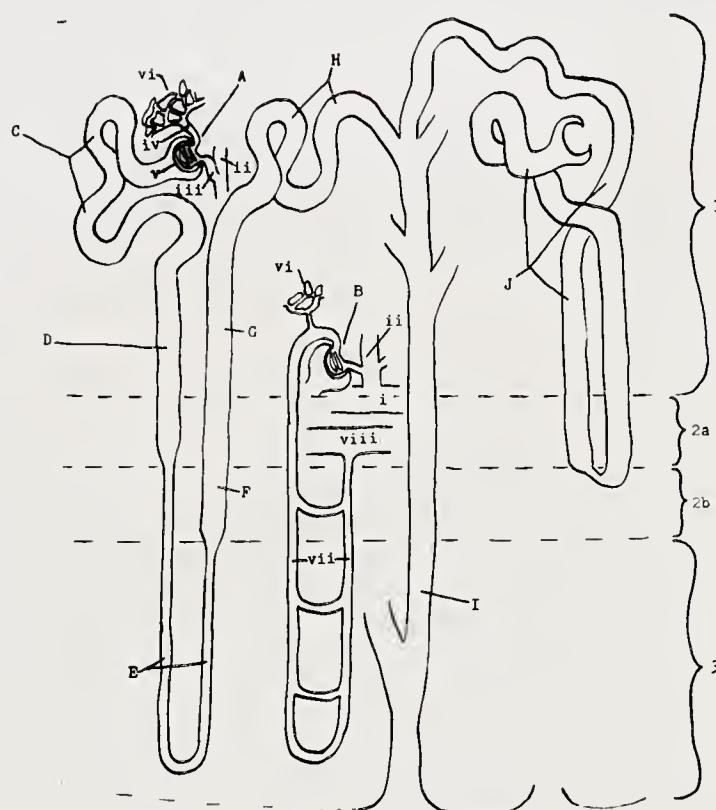
Renal Zones

1. Cortex   2. Outer Medulla   2a. Outer Strip   2b. Inner Strip   3. Inner Medulla

Nephron Unit

A. Cortical glomerulus B. Juxtamedullary glomerulus   C. Proximal convoluted tubule

D. Pars recta   E. Thin loop of Henle   F. Thick ascending loop of Henle

G. Intercalated segment   H. Distal convoluted tubule   I. Collecting Duct

J. "Cortical Nephron"

Blood Supply

i Arcuate artery   ii Interlobular artery   iii Afferent arteriole

iv Efferent arteriole   v Glomerular tuft   vi Peritubular capillary network

vii Vasa recta   viii Arcuate vein

and flow into the arcuate vein. These loops are known as
vasa recta and are the sole blood supply to the medulla.
The arcuate veins join to form larger veins as they leave the
kidney. These join together outside the kidney in most
mammals to form one renal vein which runs into the inferior
vena cava.

(d) Nerve Supply (Harmon & Davies 1948)

The blood vessels of the kidney are well supplied
with sympathetic nerves. These arise from the splanchnic and
abdominal ganglia and make up the renal plexus along the renal
artery and aorta. The nerve fibres enter the kidney with the
blood vessels and are distributed mainly to the cortex. No
evidence of nervous action on tubule cells or of vagal inner-
vation has been demonstrated.

Harmon and Davies describe two types of nerves in
the kidney and suggest that the less common type may be
afferent fibres, although this has not been proven.

3. Glomerular Filtration

Glomerular filtration is a mechanical process. The
force which produces the filtration through the glomerular
membrane is arterial blood pressure reduced to 65% of normal
by the resistance of the renal vessels (Lovatt-Evans 1956
p 1024). Opposing this reduced arterial pressure are the
forces of oncotic pressure, produced by non-filtered plasma
proteins, and transmural pressure, the pressure on the ureteral

side of the glomerular membrane. Transmural pressure is influenced by ureteral pressure, renal venous pressure, and because of the rigid capsule surrounding the kidney by such factors as increased abdominal pressure and increased cell turgor within the kidney. The magnitude of the oncotic pressure is 20-30 mm Hg and the transmural pressure is probably very close to the interstitial pressure in the kidney of 20-30 mm Hg (Swann et al. 1951).

According to Smith (1951 p 53) the glomerular filtration rate is very constant over wide physiological ranges of urine production suggesting that the regulation of urine volume is a tubular function accomplished by reabsorption of varying amounts of filtrate from the tubule lumen and not a glomerular function. The glomerular filtration rate has also been reported to remain amazingly constant over wide physiological ranges of arterial pressure. Shipley and Study (1951) varied the perfusion pressure from an average of 80 to 180 mm Hg in semi-isolated dog kidneys without changing the GFR. They attributed this property to the architecture of the blood vessels in the kidney and called it autoregulation. However, in the same year Thomson et al. found that decreasing the arterial pressure perfusing the kidneys in situ by using intra-aortic occlusion or haemorrhage, invariably reduced the GFR. Winton (1956) thinks that autoregulation does occur in kidneys, and discusses several theories of its mechanics, but he states that no theory

explains all the facts about this phenomenon.

Increasing the renal venous pressure decreases the GFR (Selkurt et al. 1949, Blake et al. 1949). The mechanism is not well understood, but is probably due to increased intrarenal pressure transmitted to the ureteral side of the glomerular membrane. Hwang et al. (1950) showed that the decrease in GFR produced by an increase in renal venous pressure was not sustained in dogs, since two weeks after an operation which chronically raised the renal venous pressure the GFR had returned to near normal values despite the maintained increase in venous pressure. Arterial pressure changes were not measured in these experimental animals.

4. Urine Concentration

Urine concentration is accomplished by removal of water from the tubule contents without concomitant removal of solutes. Enzymatic activity is assumed to be involved in this process since poisoning of the kidney with cyanide (Smith 1951 p 344) results in the production of isotonic urine. In 1933 Edwards postulated that the urine was concentrated by active transport of water from the distal tubule without transport of solute. Evidence supporting this theory is presented by Smith (1951). Active transport of water has not been demonstrated in any animal systems and water movements are normally accomplished by transport of solute with the concurrent passive movement of water. A mechanism of this

type also requires that a high osmotic gradient exists across a single layer of epithelial cells, which is not an impossible situation, but is not ideal from a teleological view.

A theory of urine concentration utilizing the hair-pin countercurrent multiplication principle does away with these objections and gives the peculiar arrangement of the loops of Henle and the vasa recta outflow an important role in urine concentration. Because this principle does not appear to be well understood even by renal physiologists a description of it and a related principle, countercurrent exchange, is given below.

(a) The Hair-pin Countercurrent Mechanisms

(i) The Countercurrent Multiplication Principle

This principle was formulated by Kuhn and Martin in 1941. It has several applications in industry where it is desired to produce high concentrations or gradients by multiplication of a small effect. The gas centrifuge (Kuhn & Martin 1941) is one application.

The application of the principal to renal concentrating mechanisms was suggested by Hargitay and Kuhn in 1951. If two spaces in a container separated by a semipermeable membrane are filled with solutions of equal concentration there will be no net flow of solute or solvent between them and their concentrations will remain the same. If, however, hydrostatic pressure is applied to one side only, solvent will be forced into the adjoining compartment, diluting it, while the

first compartment will be concentrated somewhat. Thus an
osmotic pressure difference will exist between the two compart-
ments equal in size to the applied hydrostatic pressure and
opposing it. Its magnitude is calculated by this equation.

$$\Delta p = (C_1 - C_2) \; RT \tag{1}$$

where Δp = osmotic pressure, C_1 and C_2 = final concentration on
the two sides, R is the gas constant and T the absolute
temperature.

By use of the same magnitude of hydrostatic pressure
a more concentrated solution can be produced by taking the more
concentrated solution produced during the initial procedure
and repeating this procedure upon it. It can be seen that
after several steps one would have a small volute of concen-
trated solution.

This static system can be represented dynamically in
the following way. A long tube has a partition along its
length which divides it into two compartments (Fig. 2). A
capillary tubing at end B joins the two compartments and is
of such dimensions that the resistance to flow in the larger
tubes is negligible. A dilute solution is led into the upper
compartment at A. The hydrostatic pressure causes solvent to
flow into the lower compartment until an osmotic pressure
difference between partition 1 and 2 of a magnitude which can
be calculated by equation (1) is reached. However, since
fluid flows from A to B in the upper tube its contents will

Figure 2 - Countercurrent Multiplication

Hydrostatic pressure head

Third compartment

A

B

Semipermeable membranes
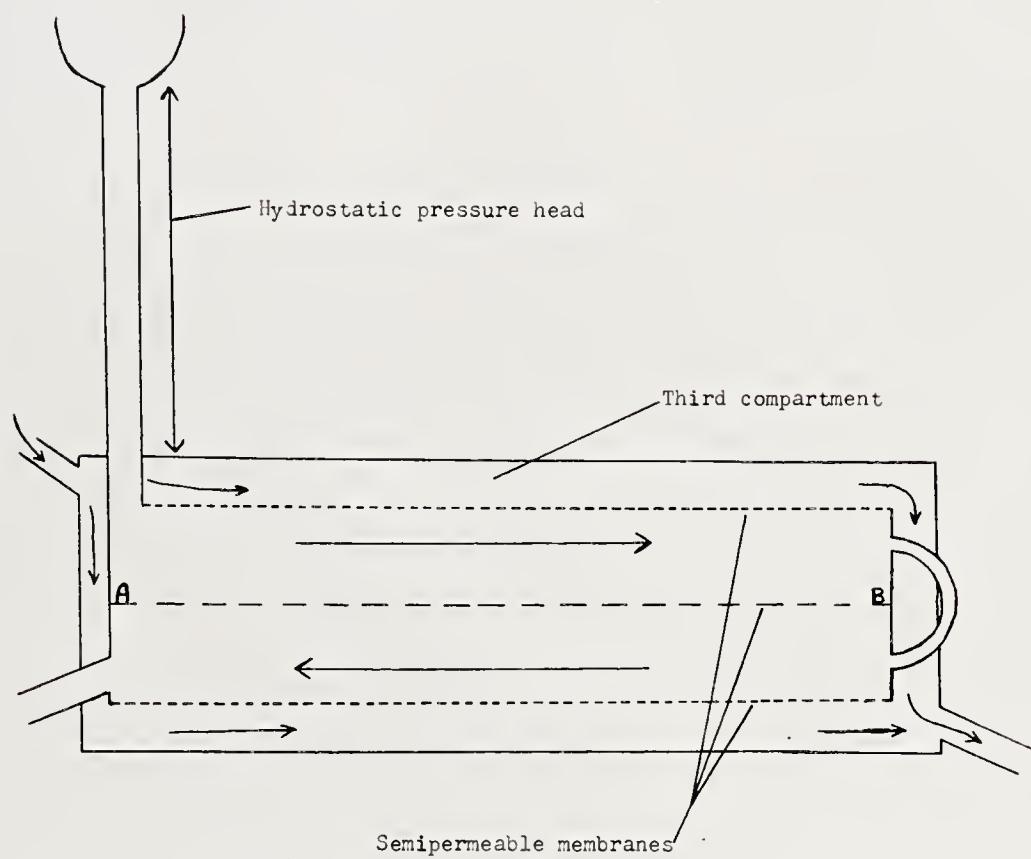
become more and more concentrated during its passage and
this single concentration effect becomes multiplied down the
length of the tube. On the passage from B to A in the lower
tube the contents become diluted again by the solvent which
has been filtered through the semipermeable membrane. Thus a
high concentration gradient has been produced between A and
B by addition of an infinite number of small concentration
gradients produced along the way. The solvent, in effect, has
been short-circuited through the beginning of the loop and
the solute has been forced to travel the full distance.

Hargitay and Kuhn (1951) showed by mathematical
calculations that the concentration in the upper tube at
any point in such a system could be calculated from this
formula:

$$C_X = \frac{C_O}{1 - \dfrac{\gamma\, P}{au} X} \qquad (2)$$

X is the length of the tube
C_X is the concentration at the end

C_O is the concentration at the beginning

γ is the permeability of the membrane
p   is the pressure applied
a   is the width of the compartments
u   is the velocity of flow

Implications of this formula will be discussed
later. It should be remembered that although the gradient
along the tube may be considerable, the gradient across the

membrane at any point will always be the same and can be calculated by equation (1). The osmotic difference between the fluid entering the system and that leaving is of this magnitude also.

The system just described has no provision for removal of concentrated solutions, since the fluid emerging is even more dilute than that which entered. However, concentrated solutions can be produced by allowing partial equilibration between the two arms of the loop and a third compartment which is separated from them by a semipermeable membrane (See Fig. 2). The fluid in this compartment is drawn off as it reaches the concentrated end of the loop (B). The extra solvent which enters the countercurrent mechanism reduces the gradient which is reached along its length. However, provided the flow velocity of the third compartment is slower than that of the loop and/or the permeability of the semipermeable membrane separating it from the loop is less than that of the one between the loops, a substantial gradient can be produced.

An analogy can be drawn between the foregoing description and the anatomy of the kidney. The concentration loop corresponds to the loops of Henle and the third compartment is analogous to the collecting ducts.

(ii) Countercurrent Exchange Principles

This description is based on an article by Berliner et al. (1958). Countercurrent exchange is used to <u>maintain</u> an existing gradient rather than to <u>produce</u> one. One of its

many applications is in airconditioning systems where the incoming fresh air is drawn through a mesh of pipes containing stale outgoing air. This maneuver tends to equalize their temperatures, saving on heat or refrigeration expenses. The exchange process is passive and the efficiency of exchange varies inversely with the flow rate and is directly proportional to the transmittance of the dividing material between the two phases and the length of time the two phases are in contact. Thus the equilibration which occurs between the third compartment and the concentration loop, as described in the previous section is countercurrent exchange.

The foregoing description applies to two separate streams, flowing in opposite directions. Countercurrent exchange can also occur within the same stream if it is suddenly turned back upon itself so that two sectors of the same stream are equilibrating with each other. This application will be discussed later.

It should be remembered in considering these mechanisms that countercurrent multiplication is an active process requiring energy, whereas countercurrent exchange is passive and requires no energy.

(iii) Evidence Confirming Countercurrent Multiplication as the Mechanism of Urine Concentration

If countercurrent multiplication is responsible for urine concentration, two criteria should be met:

(1)   The osmotic pressure of the urine at the end of the
proximal convoluted tubule should be higher than that of the
urine at the beginning of the distal convoluted tubule;

(2)   The osmotic pressure of the contents of the papilla
should increase towards the tip and should be equal to or
higher than that of the urine being produced.

Walker et al. reported in 1941 that they had obtained
three samples of urine by micropuncture from the distal con-
voluted tubules of rats. Two of these samples were hypotonic
and the other was isotonic. They also showed that fluid in
the first 2/3 of the proximal convoluted tubule was isotonic.
All animals studied were producing hypertonic urine. Wirz
in 1957 reported on tubular urine samples from eight thirsted
rats producing hypertonic urine. He found that samples drawn
from the beginning of the distal convoluted tubule were
hypotonic and samples drawn from the end were isotonic. In
1958 Gottschalk and Mylle published a short note confirming
these results and in 1959 a longer paper was published in
which they reported on the osmolality of tubular urine samples
obtained by micropuncture along the renal tubules of various
rodents which were producing hypertonic urine with or without
an osmotic diuretic. Without exception all proximal convoluted
tubule samples were isotonic with blood, all samples from the
beginning of the distal convoluted tubule were hypotonic and
those from the end were isotonic. In addition they found that
the contents of the bends of the loops of Henle, the vasa recta

and the collecting ductswere equally hypertonic at the same level.

The earliest recorded observations of the hyper-tonicity of the medulla were probably made by Hirokawa (1908). He measured the weight gain, due to uptake of water, of tissue slices placed in solutions of increasing osmolality, and found the medulla slices continued to gain weight in stronger solutions than the cortical slices. He found that medullary slices obtained from animals producing the most concentrated urine had to be placed in the most concentrated solutions before they stopped gaining weight. Wirz et al. confirmed these findings in 1951 by microcryoscopic methods. They measured the freezing point depression of slices of kidney 30 μ thick cut at right angles to the long axis of the papilla. They found that the osmolality throughout the cortex remained constant and approximately equal to plasma, but it rose continuously in the medulla towards the tip of the papilla.

This increased osmolality is presumably due to several compounds. Oliver (1921) showed that urea was concentrated in the medulla. Glimstedt (1943) and Ljundberg (1947) found increasing Cl ion concentrations in the medulla towards the tip, especially in the inner strip and the outer portion of the inner zone. In 1956 Ullrich and Jarausch showed that the concentration of urea, Na ion, Cl ion and exogenous creatinine all increased sharply in the outer medullary zone in dogs. In animals producing concentrated urine this rise

continued, in the inner zone at a slower rate towards the tip
of the papilla. In those in a state of water diuresis, the
concentrations rose only slightly in the inner zone. Thus,
since the two criteria are met, there is good reason for
accepting the countercurrent principle as the mechanism of
urine concentration. A theory of the mechanics of concentration
is presented later in this section.

(b) Factors Influencing Urine Concentration

(i) Antidiuretic Hormone (ADH).

Antidiuretic hormone is secreted into the blood by
the posterior pituitary. It is a cyclic polypeptide, differing
slightly in structure between species.

Although urine concentration can occur in its absence
(Del Greco and De Wardener 1956, Berliner and Davidson 1957)
it is thought that it is normally responsible for production
of hypertonic urine. Its release is triggered by stimulation
of osmoreceptors in the hypothalamus which respond to hypertonic
solutions (Verney 1947, 1948). These receptors cause the cells
of the posterior pituitary to release ADH and disruption of
this pathway or obliteration results in a condition of
extreme polydipsia and polyuria known as diabetes insipidus.

The action of ADH upon the urinary tubules has not
been demonstrated directly. Investigations by Koefoed-Johnsen
and Ussing (1953) on the isolated frogskin showed that the
application of ADH increased the net flux of water flowing
through it due to an osmotic gradient. Ussing and Zehran

(1951) found that ADH also increased the movement of Na ions in the frogskin. Leaf et al. (1958) found the same thing with the isolated urinary bladder of the toad. This is indirect evidence that ADH makes the renal tubules more permeable to water and perhaps to Na ions. If this is true its effect on urine concentration would be equivalent to increasing δ in equation (2).

Ginetzinsky (1958) suggested that ADH acted to make the renal tubules more permeable by causing the release of hyaluronidase from the cement substance between the tubule cells. He based this hypothesis on the observation that hyaluronidase is eliminated in the urine in the presence of ADH and is not in its absence. He also claimed that the metachromism of the cement substance is altered from a state of water diuresis to thirst and published histological figures showing this.

(ii) Urine Flow (V)

Robinson (1954 p 76) states that "maximal urinary concentration can only be attained at minimal rates of flow." There is much evidence for this. Very early investigations by Dreser (1892) and Galeotti (1902) showed that infusions of a large amount of 10% NaCl into rabbits or dogs respectively produced a diuresis of very dilute urine in animals which had previously been producing small amounts of very concentrated urine.

Much later McCance (1945) rediscovered this phenomenon in man. Further experiments (Hervey et al. 1946, Rapoport et al. 1949 a and b) showed that the amount of dilution produced by various osmotic diuretics was related entirely to the minute volume and not to the type of osmotic diuretic used. Examination of equation (2) shows that as "u" increases the final concentration produced by the CCM system decreases. The efficiency of countercurrent exchange is also decreased by increased flow velocity. A combination of these two factors is probably responsible for this phenomenon.

Robinson (1951 p 84) has shown by calculations based on a heat transfer formula that the failure to concentrate at high flows cannot be attributed to incomplete equilibration between dilute urine in the axial stream and concentrated urine adjacent to the tubule surfaces, since complete equilibration occurs with any conceivable dimension of kidney tubule.

(iii)   GFR

In 1956 Del Greco and De Wardener found that animals undergoing a "water" diuresis could produce hypertonic urine instead of hypotonic urine if the solute load to the tubules was reduced by reducing the GFR. Berliner and Davidson (1957) duplicated these results. They showed, however, that the efficiency of the concentration was greatly reduced since the amount of water required to restore the urine to isotonicity was about 0.2-0.3 ml/minute. In a normal animal reacting normally to ADH, this value may reach 5 ml/minute. Further

studies by Levinsky et al. (1959a) on hydropenic dogs under-
going ADH induced antidiuresis, showed that reductions in
GFR to 70% of normal caused an increase in the concentration
of the urine above what theoretically should have been the
"osmotic ceiling" (Robinson 1954 p 78). Decreases in GFR
to less than 70% of normal invariably resulted in production
of less concentrated urine even though the volume was smaller.
In some cases a reduction of only 10% caused less concentrated
urine to be produced.

(iv) Urea

Urea is a non-toxic, highly soluble compound which
is the main end-product of degraded protein in mammals. Urea
is the main osmotic constituent of urine and its rate of
excretion is related directly to the minute volume, as was
shown by Shannon (1936). This has been confirmed by numerous
investigators.

Recently Levinsky and Berliner (1959 b) showed
that the urine concentrating ability of dogs was impaired by
maintaining them on a low protein diet. Oral administration
of small amounts of urea increased Uosm by 25%. Under these
circumstances V also increased by about 100%.

Another property that has been noticed for a long
time is the ability of animals to excrete more concentrated
urine after the administration of urea as an osmotic diuretic
than after the administration of other solutes. Gamble et al.
(1934) probably did the first studies on this and Gottschalk

and Mylle published a paper in 1959 in which similar findings
were reported in rats after the administration of various osmotic
diuretics. Raisz et al. (1958) reported similar results in
human subjects.

(v) Renal Anatomy

Sperber (1944) attempted to relate environment to the
anatomical characteristics of the various animals' kidneys
that he measured. In general, he found that animals which
lived in dry environments and animals which ate a great deal
of protein (i.e. carnivores and insectivores) tended to have
longer loops of Henle and a greater proportion of long loops,
than water-dwellers or herbivores. These animal-types also
have longer thick ascending loops of Henle.

Sperber also found that the glomeruli closest to the
cortico-medullary division (juxtamedullary glomeruli) have
loops which extend the furthest into the medulla, often down
to the area cribrosa. The possible relation between this
fact and the fact that the efferent artioles of the juxta-
medullary glomeruli form the vasa recta will be discussed later.

(c) A Theory of the Mechanics of Concentration

The theory presented here is not unique. Its
fundamental mechanisms were postulated by Ullrich (1960).
Berliner et al. (1958) suggested the function of the vasa
recta. The only new suggestions are that a dual countercurrent
multiplication exists in the concentrating kidney, ADH is
given a more diversified area of tubule epithelium to act

upon and the peculiarities of the juxtamedullary glomerular outflow are integrated into the general scheme and given a significant role in the efficiency of the concentrating system.

Investigations of Ullrich and Jarausch (1956) showed that tissue concentrations of NaCl and urea increased most rapidly in the outer medulla as analysis was made of kidney sections cut parallel to the tip of the papilla. The largest increases occurred in the inner strip. They concluded from this that an active transport system capable of reabsorbing NaCl and possibly urea, without reabsorption of water, was present in the thick ascending loop of Henle. They suggested that countercurrent multiplication occurred between the thick ascending and thin descending segments of the loop in this vicinity and that in the presence of ADH the collecting ducts became permeable to water and equilibrated with the hypertonic interstitium here and also in the inner medulla. They regarded the portion of the loop in the inner medulla as an extension of the concentration area which allowed more complete equilibration with the collecting ducts to occur.

However, in concentrating kidneys they found that the increase in NaCl and urea concentrations continued to the tip of the papilla although the rate of change in concentration was not as much as in the inner strip. This did not occur in kidneys producing dilute urine. Gottschalk and Mylle (1959) found that the osmolality of the urine was highest in the bends of the thin loops which were closer to the papillary tip.

They suggested that concentration occurred along the full length of the loop, but also stated that the increased concentration could have been due to solute transported to the inner medulla from the inner strip by the vasa recta. This latter idea was presented by Berliner et al. (1958) in an attempt to explain the findings of Ullrich and Jarausch (1956).

It appears just as reasonable to suggest that the epithelium of the thin loop may be made more permeable to water by ADH in the same way that the collecting ducts are. This is not unreasonable since, except for thickness, the epithelium of the thin loop of Henle is similar to the clear epithelium of the collecting ducts. If this were the case, then further concentration could be produced in the thin loop by ultrafiltration as in the model described previously. The pressure head would be provided by the slight pressure gradient which causes urine to flow through the tubule.

This would provide a small auxiliary concentrating mechanism which was mechanical in nature as well as the more important enzymic system described by Ullrich.

Ullrich assumes that in a concentrating kidney the hypotonic urine which is produced at all times in the thick ascending part of the loop by selective reabsorption of solute, is reduced in volume and made isosmotic by passage of water into the peritubular network, in the distal convoluted tubule which is permeable to water due to the action of ADH. Because of the

similarity in structure between the intercalated segment and the collecting duct epithelium it seems most reasonable to assume that the ADH acts mainly on this part of the distal convoluted tubule and that the water leaves here. This is not, however, a major point in the functioning of the system.

The role of the vasa recta in this system is probably the same as that of the peritubular network, namely, to remove reabsorbed water and solute from the interstitium and to supply oxygen to and remove metabolites from the tubules. Its looped structure enables it to act as a countercurrent solute exchanger in the medullary region and thus it does not destroy the solute gradient produced, as much as it would if the blood flow was one way. Some solute of course is probably removed in the normal course of events, due to incomplete equilibration of the exit limb with the entry limb. The amount of solute removed would depend on the rate of flow and at very low flows complete equilibration would be most likely to occur.

None of the authors mentioned seems to have noted that the juxtamedullary glomeruli play a very important part in the efficiency of the concentration system. They have the longest loops according to Sperber and, therefore, function of their concentration mechanism is necessary for production of the most concentrated urine. Since their outflow is the sole blood supply to the medulla, they control the oxygen supply to

the carrier systems necessary for solute reabsorption in the thick loop. Slight interference with juxtamedullary glomerular flow would probably increase urine concentration because the vasa recta would equilibrate more fully and remove less solute from the medullary region making concentration more efficient. Greater interference, however, would probably result in more dilute urine being produced because of oxygen lack to the enzyme carrier systems and because the longest loops would no longer be efficient.

The sequence of events in a concentrating kidney could be as is shown in Figure 3.

Figure 3 - The Mechanics of Urine Concentration

H₂O movement →
active solute movement ⬛
passive solute movement ⇨

Figure 3:
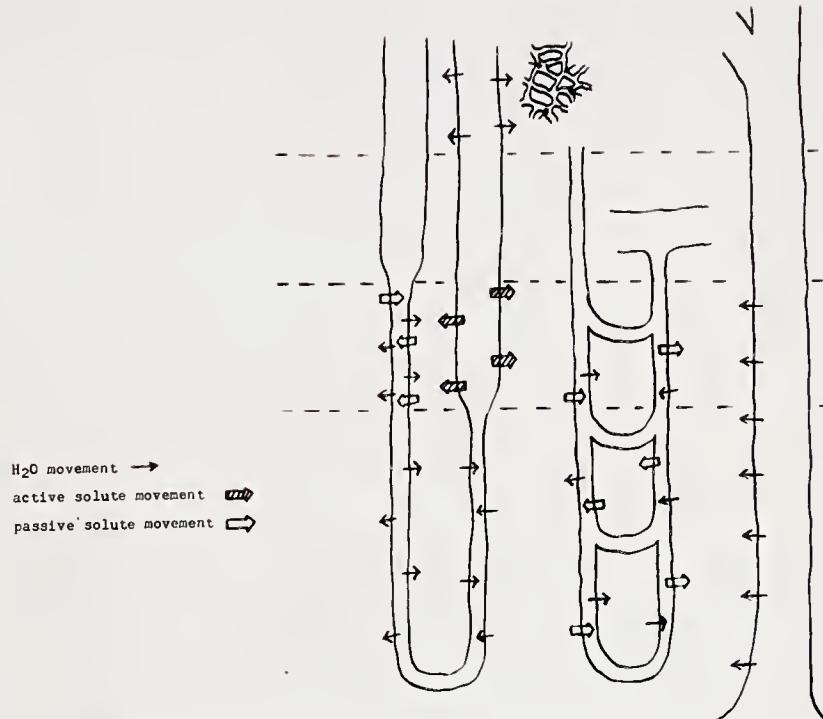
Isotonic urine enters the thin loop of Henle, after having been reduced to 15% of the volume of the glomerular filtrate by isosmotic reabsorption from the PCT. In the first part of the thin loop the urine becomes hypertonic by equilibrating with the hypertonic interstitium produced by active reabsorbtion of solute from the lumen of the ascending thick loop. This urine is further concentrated in its passage down the thin loop by ultrafiltration through the walls of the tubule. The water which leaves enters the loop again, rediluting the concentrated urine in the thin ascending loop. The urine becomes very hypotonic in the water impermeable thick ascending loop due to active removal of solute, but water again leaves the tubule later in the intercalated segment because ADH has made it water permeable also. The water which leaves enters the peritubular capillary network and the urine becomes isotonic again. The pH is adjusted in the distal convoluted tubule and the cortical part of the collecting duct. When the urine flows through the medullary part of the collecting duct water leaves and enters the hypertonic interstitium because ADH has made this part of the collecting duct permeable to water also. The final concentration of the urine depends on the amount of equilibration which occurs.

The system just outlined will produce hypotonic urine if we assume that the thin loop of Henle, the intercalated segment and the medullary portion of the collecting duct become relatively impermeable to water in the absence of ADH. Some water must be able to pass through them at all times because hypertonic urine can be produced in the absence of ADH at very low urine flow velocities (Del Greco & De Wardener 1956).

CHAPTER THREE

EXPERIMENTAL PLAN AND GENERAL METHODS

Part I - The Experimental Plan for Studying the Role of the Concentrating Mechanism in Producing Oliguria

The general preparation of experimental animals used for this investigation was almost identical to that used by Van Petten. The major difference was that antidiuretic hormone (Pitressin) was administered with the constant infusion of the clearance compounds to ensure that the concentrating mechanism was operating maximally under the conditions of the experiment. In addition to this an osmotic diuretic, urea, was administered from the beginning of each experiment to provide ample urine for clearance studies. In all but two experiments, dogs #1 and 2, an attempt was made to maintain the blood volume at a constant level by an intra-arterial injection of Dextran, equal in volume to the amount of blood withdrawn in the arterial and venous samples, and given immediately each time after the samples were obtained.

It seemed likely that increased venous pressure was responsible for the oliguria, so measurements of the increase produced by meter installation were made by inserting a catheter into the left gonadal vein and placing its tip just inside the renal vein. It was decided to increase the venous pressure after insertion of the meter to see if the magnitude of the oliguria increased. Since work by Haddy et al. (1958) suggested that the effects of increased venous pressure could be mechanical and/or reflex the peripelvic regions of both kidneys were infiltrated with local anaesthetic before and

after the pressure maneuvers in an attempt to separate the two effects.

For statistical purposes, four treatment periods were used; the control period (C), increasing venous pressure after meter installation (P), installation of the meter (M), and infiltration with local anaesthetic (A). These were randomized so that variance analysis for significant differences could be used, and to provide as much information as possible from a minimum number of experiments. Since the control period had to come first and increasing the venous pressure could be done only after installation of the meter, only the following randomizations were possible: CMPA, CMAP and CAMP.

The functional measurements made were urine volumes, GFR (clearance of creatinine) and ERPF (clearance of PAH) bilaterally and TRPF, E_{PAH} and metered RBF on the left side only. The arterial pressure and the left renal venous pressure were also measured, as well as the solute content of the plasma and of the right and left urine samples for each separate clearance period. The values used for comparison of the effects of the treatments were obtained by averaging values obtained for three consecutive ten-minute clearance periods after any particular treatment. At least 25 minutes was allowed between the time any treatment was started and the beginning of the first clearance period. This allowed conditions to stabilize and reduced errors due to "lag" in urine production.

Part II - Methods

The descriptions in this chapter are of methods used for all three series of experiments. Where slight variations occurred between experiments these are noted. The special methods used in the two preliminary series are described in the next chapter along with the results.

1. Surgical

(a) Preparation of the Animal

Dogs of either sex, weighing between 7 and 15 Kg with most weighing between 10 and 12 Kg were used. They were starved for 24 hours before the experiments, but were allowed free access to water. Anaesthesia was induced by 35 mgm/Kg of sodium pentobarbital administered intravenously or intraperitoneally. Additional anaesthetic was given intravenously as required to maintain stage III plane i level of anaesthesia.

An electrosurgery unit was used to make all skin incisions and for cauterizing severed blood vessels. The right jugular vein was used to pass a pickup catheter into

the left renal vein. The left jugular was used for admin-
istration of the constant perfusion and also returned renal
venous blood to the main circulation after it had passed
through the meter. A catheter passed into the abdominal
aorta from the femoral vein was used to obtain systemic
arterial pressure and also for arterial blood samples.

A midline laparotomy extending from the xiphi-
sternum to just above the pubis was performed. The ureters
were separated from the dorsal body wall on either side of the
colon where they cross the iliac arteries. They were tied
off close to the bladder and canulated with small polyethylene
catheters. They were often cut through completely after the
canulation to prevent kinking. If the bladder was full the
urine was removed with a syringe.

The left renal vein and left gonadal vein were
exposed by following up the right side of the dorsal mesentery
and gently packing the intestines to the left side of the
abdominal cavity. The left renal vein was freed by blunt
dissection close to its union with the inferior vena cava
and a tie was passed around it with an aneurysm needle. The
left gonadal vein was catheterized using a large bore needle
with a polyethylene catheter inside it. Renal venous pressures
and blood samples were obtained here. After the meter was
installed renal venous samples were obtained from a withdrawal
diaphragm on it. This catheter was also used for administration
of heparin and extra anaesthetic. If renal venous pressures

were not being measured the gonadal vein was tied off to prevent contamination of the renal venous blood samples.

An interval of atleast thirty minutes was allowed after the completion of surgery before control clearance studies were done.

(b) Installation of the Meter

The meter was prepared for installation by filling it with heparinized normal saline (5 mgm %). Antifoam was sprayed into the bubble trap and the outflow was connected to the catheter in the left external jugular vein. A 16 french drainage catheter about 30 inches in length was inserted into the right external jugular vein and was passed through the right heart and into the inferior vena cava with the assistance of a stiff, slightly curved metal obturator, placed inside it. When the tip of the catheter was inside the abdomen the obturator was partially withdrawn and the catheter was maneuvered gently into the left renal vein by hand and secured there with the tie previously placed around the vein. The obturator was then withdrawn completely and the catheter was clamped until it was connected to the inflow side of the meter.

The blood was rendered incoagulable before the meter was installed by intravenous administration of heparin (4 mgm/Kg). Maintenance doses of 5-10 mgm were administered every half hour. The meter used was identical to that described by Nash and Milligan (1959).

(c)   Anaesthetizing the Kidneys

The kidneys were anaesthetized by infiltration of 3-4 ml of 1-2% xylocaine solutions or a mixture of half 1-2% xylocaine and half 0.1% nupercaine was used when a longer period of anaesthesia was desired. Infiltration was done on each side of the hilus of the kidney in the peripelvic region. These two local anaesthetics were used because they will not interfere with PAH determinations and they are non-toxic in the concentrations used.

Observations showed that anaesthesia usually lasted for one hour with xylocaine alone and two hours with the mixture. When necessary maintenance injections of xylocaine only were given. Injecting xylocaine around the origins of the renal arteries was tried but was not very successful.

The amount of anaesthesia at the end of the experiment was judged very roughly by rapidly exsanguinating the animal through the renal venous catheter. Observations on unanaesthetized kidneys showed that under these conditions flow stopped entirely when the systemic pressure fell below 50-60 mm Hg. In all the anaesthetized kidneys, blood flow continued until the systemic pressure fell below 35 mm Hg or lower, which was taken to indicate that most or all of the nerve supply to the blood vessels was blocked.

(d)   Adjusting the Renal Venous Pressure

The renal venous pressure was increased by partial occlusion of the pickup catheter where it emerged from the neck. Attempts were made to increase the venous pressure as much as possible with minimal interference with the RBF as measured by the meter. The pressure could usually be raised 10-15 mm Hg without large decreases in blood flow, but increases to over 35 mm Hg invariably reduced the RBF substantially.

2.   Measurements of Renal Functions

(a)   Renal Clearances

(i)   Theory

Ficks' principle, Volume X Concentration = Amount (1) is the basis of renal function tests. It can be rewritten as

$$\text{Volume} = \text{Amount/Concentration} \tag{2}$$

If a time factor is incorporated into this equation it becomes

$$\text{Volume/unit time} = \frac{\text{Amount/unit time}}{\text{Concentration}} \tag{3}$$

Thus if the amount of some substance excreted by the kidney in a given length of time is known and its concentration in the plasma is also known, the volume of blood cleared of this substance (C) can be calculated by using equation (3). If the substance is completely removed from the blood during one passage through the kidney, the calculation gives the blood flow through the kidney per unit time. If the substance is not entirely excreted by one passage through the kidney the clearance (C) can be corrected to give the blood flow if the change in

concentration is known. Thus, if the plasma concentration of the substance is reduced by 80% the clearance may be assumed to be 80% of the total blood flow.

If a substance is not synthesized, destroyed, or stored in the kidney and is neither secreted or reabsorbed by the tubules, its clearance may be used to measure glomerular filtration rate (GFR), since it can be removed from the blood passing through the kidney only by filtration.

In practise the amount of substance present in the urine is calculated by determining its concentration (U) and multiplying by the volume per unit time (V). Thus (3) appears as $C = \dfrac{UV}{P_a}$. P_a refers to plasma concentrations and this formula gives plasma clearances. If clearance of blood is desired it can be determined by correcting with the hematocrit value. $C \text{ (of blood)} = \dfrac{C \text{ (plasma)}}{1 - \text{hematocrit}}$

In these experiments, the clearance of creatinine was used to measure GFR, and the clearance and extraction of PAH were used to measure renal plasma flow (RPF). The extraction of PAH by the kidneys is usually greater than 80% and PAH clearance is referred to as effective renal plasma flow (ERPF).

(ii) Maintenance of Adequate Urine Volume.

To reduce systematic errors in clearance determinations referable to small urine volumes and to reduce the time required for flushing out the renal "dead" space between experimental maneuvers, it was decided to administer an osmotic

diuretic. In the two preliminary series of experiments a priming dose of 20% mannitol 2 ml/Kg was used followed by administration of 0.65 ml/minute of a 10% solution of mannitol. In the last series when concentrated urine was desired, urea was substituted for mannitol and vasopressin was administered with it at the rate of 0.5 mu/Kg/minute. This usually provided at least 2 ml of urine per ten minute collection period and U/P ratios varied from 2 to 3.

(iii) Maintenance of Plasma Levels of PAH and Creatinine

Another factor which is necessary for accurate clearance values is stable levels of the clearance substances in the blood. This is accomplished by administering a priming dose of the substances to produce the required plasma levels and then maintaining these by replacing the amount excreted by using a continuous controlled drip or constant infusion.

According to Homer Smith (1951 p 183) plasma levels of creatinine between 7 and 15 mgm % are sufficiently high to reduce error from endogenous chromagens to an insignificant level. PAH, which has no counterparts in normal blood constituents gives optimum results at levels between 0.7 and 2.5 mgm %. Below these limits, the errors of measuring plasma concentration become very significant and the tubular excretion limit is usually reached at plasma levels of 4 mgm %.

The amount of the priming dose and the concentration of the constant infusion were calculated on the volume of distribution within the dogs' body tissues and the average rates

of excretion in the dog for these substances respectively. The percentage volumes of distribution of creatinine and PAH are 50% and 30% respectively (Greenberg et al. 1952). The average rates of excretion are dependent upon the average clearance values for these two substances which are 4.29 ml/minute/Kg for creatinine and 13.5 ml/minute/Kg for PAH. The drip was administered at a rate of about 0.675 ml/minute. A plasma concentration of 1 mgm % was calculated for PAH and of 13.5 mgm % for creatinine. There is no tubular excretion limit for creatinine since it is removed only by filtration. It was felt that a higher level would produce much less error. The general formulae for the calculations are as follows:

$$\text{Priming Dose (mgm/Kg)} = \frac{\text{\% Volume of Distribution X Plasma Conc(mgm\%) X 10}}{100}$$

This gives a factor of 30 mgm/Kg for PAH and 67.5 mgm/Kg for creatinine. Experience showed that 4.5 mgm/Kg and 50 mgm/Kg respectively gave more satisfactory results in these experiments.

$$\text{Conc of Constant Infusion(mgm\%/Kg)} = \frac{\text{Plasma conc(mgm\%) X Clearance(ml/min/Kg)}}{\text{Rate of infusion(ml/min)}}$$

This gives 20 mgm%/Kg for PAH and 86.0 mgm%/Kg for creatinine. After the priming dose had been administered and the drip started an equilibration period of 40 to 60 minutes was allowed before clearance periods were started.

It was unusual for the levels to remain constant throughout an experiment. They usually rose during the course of the experiment. However, variations during any particular set of clearance periods (three ten-minute periods) was not usually more than 10%.

(b) Collection and Treatment of Blood Samples

Arterial samples were collected from a catheter inserted into the abdominal aorta, through the femoral artery. Ten ml. of blood were withdrawn initially to make sure that there was no intermixing with the previous sample. The sample size was usually 9 ml. Two ml. of this was used for duplicate hematocrits and the remainder was centrifuged at about 3000 rpm in a clinical centrifuge at room temperature to separate the plasma from the cells. The interval between withdrawal and centrifugation was variable.

Venous samples were collected from the catheter inserted into the gonadal vein or, when the meter was installed, from a withdrawal cap on the body of the meter. Adequate discards were made before sampling to prevent intermixing with previous samples. The venous samples were centrifuged immediately after withdrawal. The time interval between the beginning of sampling and the beginning of centrifuging was never more than 90 seconds and was often less than 25. The volume of the venous samples was 3-4 ml.

If the animal had not been heparinized, the samples were withdrawn into a syringe which had been rinse with a

solution containing 10 mgm/ml of heparin, to prevent clotting.

Venous samples were centrifuged for five minutes, arterial samples for ten. After centrifuging was complete 1 ml aliquot samples were withdrawn for chemical analysis.

Blood sampling was done within an interval of two minutes in the middle of each ten minute urine collection period. In some early experiments blood samples were taken five minutes before and five minutes after the start of each urine collection period. The results were averaged later when calculations of renal function were being done. This method was discontinued when it was seen that the levels changed only slightly during this interval, and the slight increase in accuracy was not worth the extra work.

(c) Collection and Treatment of Urine Samples

Separate urine samples from each kidney were collected for accurately measured ($\pm$ 5 sec.) ten minute periods into conical, graduated, glass centrifuge tubes. In cases where blood cells were present in the urine, due to damage of the ureteral lining, the tubes were centrifuged and twice the packed cell volume was deducted from total volume.

After the urine had been collected it was diluted in 100 ml volumetric flasks, according to the following method:

Total volume	Volume taken for Dilution
Less than 1.5 ml	0.5 ml
1.5 - 4.0 ml	1.0 ml
4.0 - 10.0 ml	2.0 ml
More than 10.0 ml	3.0 ml

This scheme reduced analysis errors, since there was always an adequate concentration of material to assay. (Larger volumes of urine tend to be very dilute.) One ml aliquots were taken from this primary dilution and diluted to 20 ml for PAH determinations and to 10 ml for creatinine determinations.

(d) Chemical Analysis

(i) Precipitation of Plasma Proteins

Because the analyses are carried out at high and low pH levels it was necessary to precipitate the plasma proteins and remove them by filtration. Otherwise they would have been denatured by the chemical procedure and would have interfered with the colorimetric determinations.

Plasma samples for PAH determinations were precipitated by adding one ml of plasma to nine ml of 5% trichloracetic acid. This was shaken, allowed to stand for at least ten minutes and then filtered through #1 Whatman filter paper.

Since the filtrate for creatinine determinations had to be very close to pH 7, plasma samples for them were precipitated with tungstic acid. In the earlier experiments this was prepared by mixing distilled water, 10% Na_2WO_4, and 0.67 N H_2SO_4 in the proportions 16:1:1. One ml of plasma was added to nine ml of this solution, it was shaken and then filtered through #1 Whatman paper after it had been allowed to stand for at least 15 minutes.

In the later experiments, when urea was used as an osmotic diuretic a stronger tungstic acid solution had to be

used due to the increased ionic strength of the plasma. For
this purpose the above ingredients were mixed in the ratio
12:1:1. One ml of plasma was added to 14 ml of this solution
and it was treated as above.

(ii) Colorimetric Determination of PAH Concentration

The method used was a modification of the method of
Bratton and Marshall (1939) which was introduced by Smith et al.
(1945).

It consists essentially of the coupling of two mole-
cules of PAH to form an azo dye after reduction of the amino
groups with HNO_2. The coupling compound is N-(1-napthyl)-ethylene
diamine dihydrochloride.

The steps for the reaction are as follows:
1. To duplicate 2 ml aliquots of protein-free plasma or
diluted urine was added 0.4 ml of 1.2N HCl and 0.2 ml of 0.1%
$NaNO_2$. This produced HNO_2 in the mixture which was shaken and
allowed to stand five minutes.
2. The excess HNO_2 was removed by the addition of 0.2 ml of
0.5% ammonium sulphamate ($NH_4SO_3.NH_2$). The mixture was shaken
and allowed to stand three minutes.
3. 0.2 ml of the coupling reagent were then added, the mixture
was shaken, and its optical density was read after ten minutes
with a #54 Klett filter in a Klett photometer.

It was found most convenient to produce the reaction
in the photometer tubes themselves. A pooled plasma blank was
carried through the procedures except that water was added

instead of $NaNO_2$ to provide a zero reading for plasma. Distilled water with all the reagents added was used as a urine blank.

(iii) Colorimetric Determinations of Creatinine Concentrations.

The method used was a modification of the Folin-Wu alkaline picrate method reported by Bonsnes and Taussky (1945). The exact mechanics of this reaction are not understood, but it is though that creatinine and picric acid react in an alkaline environment to produce a highly colored complex ion.

The steps for the reaction are as follows:
1. To duplicate 3 ml aliquots of protein-free plasma or diluted urine was added 2 ml of alkaline picrate. The mixture was shaken and its optical density was read after ten minutes with a #54 Klett filter in a Klett photometer.

This reaction was also produced in the photometer tubes. The alkaline picrate was prepared immediately before it was used by mixing equal volumes of 0.04 M picric acid and 10% NaOH. This mixture is unstable and must be discarded if left more than 15 minutes before it is used. Distilled water was used as a blank for urine determinations and a plasma sample withdrawn before any creatinine was given to the dog was used as a blank for plasma determinations.

(iv) Preparation of Standard Curves

Solutions containing accurately known amount of PAH

and creatinine were prepared. Water was used as the solvent for
urine curves and the respective precipitants were used as the
solvent for the plasma curves. For urine varying amounts of
the standard solution were added to 100 ml volumetric flasks
to make concentrations that would be produced by the addition
of 1 ml of urine of a given concentration. For example :- to
duplicate the concentration produced when 1 ml of urine from a
sample with a concentration of 500 mgm % PAH, it would be
necessary to add 5 mgm of PAH to the volumetric flask. If the
standard solution contained 50 mgm % it would be necessary to
place 10 ml of it into the volumetric flask in order to get
the equivalent concentration in the final volume of 100 ml.
Creatinine concentrations were prepared in a similar manner.
The range of concentrations for PAH were equivalent to 0 - 1050
mgm %, and for creatinine to 0 - 3500 mgm %. These were on the
basis of 1 ml of urine and the concentrations had to be corrected
if the actual volume used was more or less.

A similar scheme was used for plasma curves. Varying
amounts of precipitant containing PAH or creatinine and ordinary
precipitant were combined to make total volumes of 18 and 14 ml
respectively. Two ml of dog plasma were added to the PAH
standards and 1 ml was added to the creatinine standards. The
concentrations produced were equivalent to those which would
have been produced by addition of plasma with concentrations of
0 - 3.5 mgm % PAH or 0 - 28 mgm % creatinine respectively.
Double volumes were used in the PAH determinations to reduce

systematic errors.

After the ranges had been covered, the samples were filtered or diluted as the case might be and the chemical analysis was performed. The optical density was plotted against equivalent concentrations.

The PAH color reaction follows Beer's Law and therefore gives a straight line when optical density is plotted against concentration. Creatinine, however, gives a straight line response only up to absolute concentrations of about 2 mgm %. Above this, the curve tends to flatten horizontally. In the plasma creatinine determinations this absolute concentration was never exceeded so a straight line response was obtained. For these three curves, therefore, the linear regression was calculated and the concentrations were calculated from the formula (Goulden 1952). The urine creatinine concentrations gave a curved line when plotted against equivalent concentrations so the optical density of the duplicates was averaged and a best fit curve was drawn by eye. Urine concentrations were calculated from this curve.

Whenever fresh reagent solutions were used, one or two points were checked on each standard curve to see if any gross changes were present. A variation of 10 Klett units of optical density was the maximum deviation tolerated for any check. If the deviation was greater than this, a new curve was prepared. Four representative curves and the calculations are in Appendix III.

(e)   Determinations of Solute Content of Plasma and
Urine by Measuring Freezing Point Depression

(i) Theory

Freezing point depression (FPD) is dependent upon
the number of particles of solute present in a solvent.
Calculations derived from the Clausius-Clapeyron equation show
that the freezing point of water is lowered $1.857^{\circ}C$/mole of
solute/1000 gm H_2O.   This is true with polar substances which
do not ionize.   Since biological fluids contain both polar
and non-polar substances, quantity of solute present is measured
in osmoles/1000 gm H_2O.   An osmole of solute is equivalent
to one mole of a polar or non-electrolyte substance as far as
reducing the freezing point is concerned.

(ii)   Methods

An Aminco apparatus was used.   This apparatus con-
sists basically of a cooling unit, and a combination stirrer
and temperature recorder.   The cooling unit works on the principle
of adiabatic expension.   Air, under pressure, is led through a
small aperture into a relatively large chamber, where it can
expand.   Heat is abstracted from the walls of the chamber and
from the sample reducing its temperature.   The sample is super-
cooled, and then seeded with silver iodide crystals.   As it
freezes its temperature is folowed upwards to the "plateau" or
melting point where the temperature remains constant while the
heat of fusion is being absorbed.   The melting point is of course
the same as the freezing point.   The temperature is recorded

by means of a thermister embedded in a glass stirrer which keeps the sample mixed to allow supercooling. The temperature is not measured directly, but in equivalent arbitrary, resistance units, through a wheatstone bridge arrangement and voltage measuring tube (electric eye).

Sodium chloride solutions of osmolalities ranging from 95 to 1830 m osmoles/Kg were used to prepare standard curves. Four distinct linear relationships were found over the range. Therefore, all readings within one slope interval were converted to a common equivalent and a linear regression was calculated. Resistance readings were transformed to m osmoles/Kg H_2O by means of the regression formulae obtained. Calculations and data are in Appendix IV.

(f) Measurement of Blood Pressure

Blood pressures were recorded with statham transducer strain guages on a Sanborn Twin-Viso recorder. Separate channels were used for arterial and venous pressure. The strain guages were balanced and calibrated so that pressures could be read directly from the tape. The approximate level of the inferior vena cava was taken as the zero point for each dog.

This machine is relatively stable. However, the zero point was checked frequently throughout the experiment and adjusted if necessary. Calibration was checked at the end of each

experiment. Pressures were monitored continuously throughout the experiment and recorded at the beginning and end of each urine collection period.

(g) Measurement of RBF with the Flowmeter.

Direct measurements of renal blood flow were done with an automatic recording bubble flowmeter. Details of its operation are given in the original paper, (Nash and Milligan 1959). The time marker of the Sanborn recorder was used to record the rate of flow from the meter.

It was found that replacement of the valve diaphragm changed the effective volume of the meter so a new determination of volume was made each time the valve was changed.

The blood flow was monitored continuously during the experiment and was recorded at the beginning and end of each urine collection period. The mean distance between bubble "injections" "m" was determined over a range of one minute on the tape.

3. Calculations

(a) Formulae

Effective renal plasma flow (ERPF) = Clearance of PAH

Effective renal blood flow (ERBF) = $\dfrac{ERPF}{1 - Hematocrit}$

True Renal Plasma flow (TRPF) = $\dfrac{ERPF}{Extraction\ of\ PAH}$

True Renal blood flow (TRBF) = $\dfrac{TRPF}{1 - Hematocrit}$

Extraction of PAH (E_{PAH}) = $\dfrac{\text{Arterial PAH conc} - \text{Venous PAH conc}}{\text{Arterial conc PAH}}$

Meter renal blood flow (MRBF) = $\dfrac{150 \times \text{effective meter volume}}{m}$

Glomerular filtration rate (GFR) = clearance of creatinine

True filtration fraction (TFF) = $\dfrac{\text{GFR}}{\text{TRPF}}$

% H_2O reabsorbed = $100 - \left(\dfrac{V}{\text{GFR} \times 10} \times 100 \right)$

% Solute reabsorbed = $100 - \left(\dfrac{\text{Uosm V}}{\text{GFR} \times \text{Posm} \times 10} \times 100 \right)$

Osmolar clearance (Cosm) = $\dfrac{\text{Urine osmolality} \times \text{Urine Volume}}{\text{Plasma osmolality} \times 10}$

The dimensions of all flow rates is ml/minute except urine flow which is ml/ 10 minutes. All ratios are dimensionless. Plasma and urine concentrations were calculated as mgm %. Plasma and urine osmolalities were calculated as milliosmoles/ Kg H_2O.

(b) Statistics (Goulden 1952)

The analysis of variance has been used wherever possible to determine significant differences. Variance is a parameter which is a measure of the variability of a population. Numerically it is equal to the square of the standard deviation.

Analysis of variance is a comparison of the variability of two groups rather than their mean values as in the "t" test. Since variability is involved, the effects of different treatments or combinations of treatments on the variability can be separated from each other. The variability of each of these

separate factors or groups of factors is then compared to the
residual variability, which is due to the normal variation. If
the treatment has caused no effects the variability of each of
the treatment parameters will be equal to the residual variability.
The ratio of these values is called F in honour of R. A. Fisher,
who developed an equation which determines the distribution of
F with various sample sizes. Like "t" values the F value must
be considerably larger than unity for a small number of data to
denote a significant difference.

Another factor which can be determined with variance
analysis is the effect of interaction. Interaction is the
composite effect of two or more treatments upon the variability
of the data, separated from their individual effects. The
effects of interaction cannot be determined with a "t" test.

When the variability of a group of data is significantly
different from the normal variability the factors causing this
can be determined by calculating the least significant difference
(LSD). This value is twice the standard error of the data,
calculated from the residual variance, multiplied by an
appropriate value of "t". It is similar to the fiducial limit
of a mean value. Normally the "t" value for 5% is chosen. If
two values differ by more than the LSD, they are responsible for
the significant variance of the data and are significantly
different from each other.

Regression formulas were calculated using variance.
The slope "b" was calculated by dividing the variance of the product
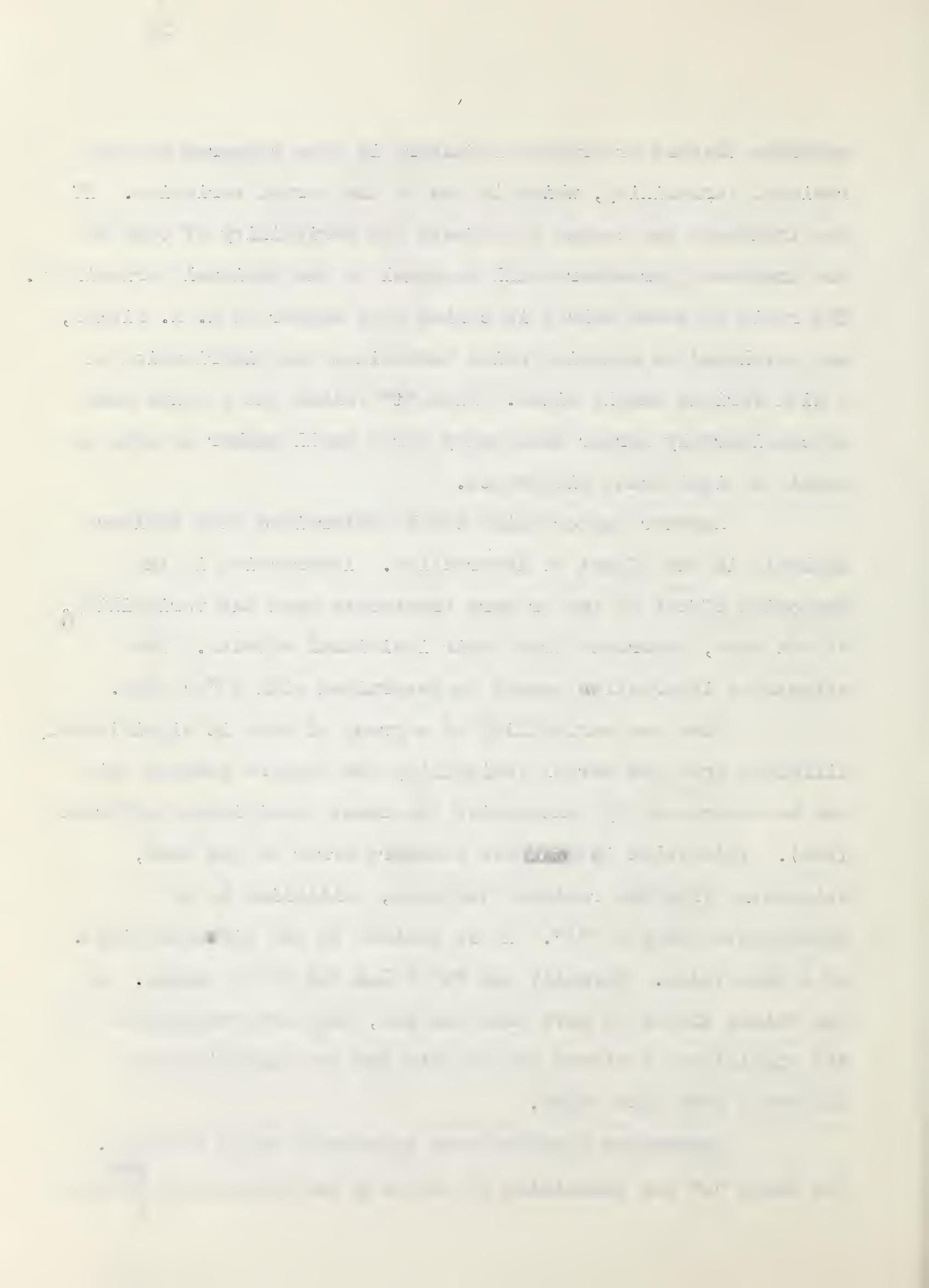
of the dependent and independent variables by the variance of
the independent variable. The intercept "a" was calculated
from the formula $y = bx + a$. The mean values of the
dependent variable "$\bar{y}$" and independent variable "$\bar{x}$" were used.

CHAPTER FOUR

RESULTS AND DISCUSSION

RESULTS

Part I - Preliminary Experiments

1.   The Effects of Storage Time and Temperature before

Centrifugation on the Plasma Levels of PAH in Dogs

These tests were designed to determine the effects

of time and temperature on both arterial and venous plasma

concentration of PAH. From this, the effects of these factors

on the calculation of E_{PAH} could also be determined. The

samples were obtained after the bubble flowmeter had been

installed under conditions close to those produced by Van

Petten. It was hoped that this would give an estimation of

the error present in his calculations.

(a) Methods

Eight tests were done on blood samples obtained

from four mongrel dogs. Blood samples were obtained by

simultaneously withdrawing 25-30 ml. of arterial and venous

blood, respectively, from an indwelling catheter in the

abdominal aorta and from the pickup catheter tied into the

renal vein which conveyed blood to the flowmeter. Each

large sample was mixed after withdrawal and was then divided

among the treatment tubes. Treatments, which were identical

for both arterial and venous samples, consisted of storage of

half of a group of similar aliquots at $30^{\circ}C$. and the other

half in an ice bath for periods of 30 and 60 minutes. The

samples stored in the cold were centrifuged after the storage

period in a refrigerated centrifuge at $0^{\circ}C$., while those

stored in the warm bath were centrifuged in a high speed

clinical centrifuge at room temperature. In addition, samples

were centrifuged in the warm and cold centrifuges "immediately"

(3-4 minutes after withdrawal). The time required to separate

the cells completely from the plasma was about three minutes

in each case. The PAH concentrations in the blood were

maintained by a constant infusion. Plasma concentrations were

determined by the method described in Chapter Three.

The error in storage temperature was negligible at

0°C., and $\pm 0.5^{\circ}$ at 30°C. The error in storage times was ± 3

minutes at 30 and 60 minutes. All the first samples were

spun within four minutes of the beginning of blood withdrawal.

The data was analyzed for significance of differences using

variance analysis. Since withdrawal of such large amounts

of blood from the dogs for each test changed the basal state

considerably each test was taken as a replicate rather than

each dog.

(b) Results (Tables 2, 3 and 4)

The results are presented in the form of analysis

tables. The treatments are coded to conserve space. The

treatment code is:

Environment   Time Interval

10 - all warm   1   immediately
20 - all cold   2   30 minutes
30 - arterial cold,   3   60 minutes
        venous warm
40 - venous warm,
        arterial cold

Table 2 - Arterial Levels of PAH (mgm/100 ml.)

Treatment	11	12	13	21	22	23	Sum of Replicates
Dog #							
10/16/59-1	0.79	0.85	0.84	0.95	0.86	0.85	5.14
10/17/59-1	1.59	1.57	1.46	1.59	1.58	1.63	9.42
10/17/59-2	1.67	1.50	1.51	1.65	1.59	1.67	9.59
10/22/59-1	1.71	1.61	1.66	1.71	1.71	1.73	10.13
10/22/59-2	1.95	1.80	1.74	1.88	1.81	1.84	11.02
10/22/59-3	2.33	2.21	2.23	2.41	2.35	2.41	13.94
10/24/59-1	1.90	1.93	1.79	1.87	1.93	1.95	11.37
10/24/59-3	2.43	2.41	2.43	2.39	2.47	2.48	14.61
Sum of Interaction	14.37	13.88	13.66	14.45	14.30	14.56	
$\bar{x}$	1.80	1.73	1.71	1.81	1.79	1.82	

Sum of Environment   10 - 41.91   20 - 43.31

Sum of Time   1 - 28.82   2 - 28.18   3 - 28.22

Total   85.22

S of V	DF	SS	MS	F	5%
Replicates	7	10.0713	--	--	--
Environment	1	.0408	.0204	1.92	--
Time	2	.0161	.00805	0.76	--
ExT (Interaction)	2	.0212	.0106	4.69	4.12
Error	35	.0792	.002262		
Total	47	10.2286			

$$LSD = \sqrt{\frac{2 \times .002262}{8}} \times 2.03 = 0.048$$

Table 3 - Venous Plasma Levels of PAH (mgm/100 ml.)

Treatment	11	12	13	21	22	23	Sum of Replicates
Dog #							
10/16/59-1	0.20	0.30	0.21	0.14	0.31	0.35	1.51
10/17/59-1	0.43	0.39	0.33	0.37	0.52	0.49	2.53
10/17/59-2	0.41	0.39	0.31	0.41	0.49	0.55	2.56
10/22/59-1	0.47	0.50	0.49	0.47	0.57	0.62	3.12
10/22/59-2	0.52	0.42	0.36	0.49	0.56	0.49	2.84
10/22/59-3	0.38	0.37	0.41	0.54	0.58	0.61	2.89
10/24/59-1	0.47	0.42	0.42	0.41	0.64	0.67	3.03
10/24/59-3	0.40	0.37	0.27	0.37	0.67	0.70	2.78
Sum of Interaction	3.28	3.16	2.80	3.20	4.34	4.48	
$\bar{x}$	0.41	0.39	0.35	0.40	0.54	0.56	

Sum of Environment   10 - 9.24   20 - 10.02

Sum of Time   1 - 6.48   2 - 7.50   3 - 7.28

Total   21.26

S of V	DF	SS	MS	F	1%
Replicates	7	.2996	--	--	
Environment	1	.1610	.1610	3.14	
Time	2	.0360	.0180	0.35	
ExT (Interaction)	2	.1029	.05145	14.58	7.20
Error	35	.1235	.003528	--	
Total	47	.7230			

$$LSD = \sqrt{\frac{2 \times .003528}{8}} \times 2.03 = 0.060$$

Table 4 - Extraction of PAH

Treatment	11	12	13	21	22	23
Dog #						
10/16/59-1	.747	.647	.750	.854	.640	.588
10/17/59-1	.730	.752	.773	.767	.672	.699
10/17/59-2	.754	.740	.795	.752	.692	.670
10/22/59-1	.725	.700	.706	.725	.667	.642
10/22/59-2	.728	.766	.793	.728	.700	.733
10/22/59-3	.834	.832	.816	.772	.754	.747
10/24/59-1	.753	.780	.765	.781	.668	.656
10/24/59-3	.845	.847	.887	.845	.728	.717
Sum of Interaction	6.116	6.064	6.285	6.224	5.521	5.452
$\bar{x}$	.765	.758	.786	.778	.690	.681

Sum of Environment   10 - 18.455   20 - 17.197

Treatment	31	32	33	41	42	43	Sum of Replicates
	.822	.635	.584	.789	.652	.753	8.461
	.767	.668	.664	.730	.753	.797	8.772
	.754	.673	.637	.752	.755	.814	8.788
	.725	.658	.627	.725	.706	.717	8.323
	.748	.688	.718	.723	.775	.804	8.904
	.768	.737	.726	.842	.842	.830	9.500
	.783	.668	.625	.748	.782	.784	8.793
	.848	.720	.712	.832	.850	.891	9.722
Sum of Interaction	6.215	5.447	5.293	6.141	6.115	6.390	
$\bar{x}$	.777	.681	.661	.768	.764	.799	

Sum of Environment   30 - 16.965   40 - 18.646

Sum of Time   1 - 24.696   2 - 23.147   3 - 23.420

Total   71.263

S of V	DF	SS	MS	F	1%
Replicates	7	.1335	--	--	
Environment	3	.0932	.03167	2.61	
Time	2	.0428	.02140	1.76	
ExT (Interaction)	6	.0729	.01215	11.68	3.06
Error	77	.0803	.00104	--	
Total	95	.4227			

$$LSD = \sqrt{\frac{2 \times .001040}{8}} \quad x \quad 1.99 = 0.032$$

Thus, treatment 12 refers to the values obtained for samples stored in the cold environment for 30 minutes before being centrifuged. Treatments 30 and 40 are used only for the extraction ratio calculations. They are included there because they increase the degrees of freedom and give a more accurate estimate of the error variance.

The column labeled "MS" gives the variance produced by each of the treatments, their interaction, and the residual or error variance.. Since we are not interested in determining if the replicates are significantly different, their variance has not been calculated.

Examination of each of the analysis tables shows that only the F value for the interaction is significant in each case. This is to be expected, since neither of these treatments can have an effect unless they interact.

The particular treatments responsible for producing the significant variance are found by determining which of the average values for the various treatments (found in the horizontal column labelled $\bar{x}$) differ from each other by more than the least significant difference (LSD).

Thus treatment in the cold produced a significant decrease in both arterial and venous plasma samples after 30 and 60 minutes respectively. The change in E_{PAH} calculated from these values were not significant however. Treatment in the warm produced no significant changes in arterial plasma levels over the 60 minute period, but venous plasma levels of

PAH increased very significantly after 30 minutes. This resulted in a very significant decrease in the E_{PAH} calculated from these values. Examination of treatments 11, 21, 31 and 41, all the "immediate" treatments for E_{PAH} shows that none of their average values differ significantly from each other. This finding is the basis of the treatment of the blood samples in later experiments as is outline in Chapter Three.

These findings agree essentially with those of Phillips et al.(1945). However, the fact that plasma levels of PAH decreased in both arterial and venous samples stored in the cold suggests that the movement of PAH out of the red blood cells in venous samples may not be due to passive diffusion, but to active transport requiring energy derived from enzymic activity instead. If this is true, and more experiments using enzyme inhibitors would be necessary to prove it, then the correction formula suggested by Phillips et al. is incorrect since it is derived by extrapolation of a graph of the speed of passage of PAH assuming passive diffusion. If enzymic reactions are involved the rate of diffusion should have been graphed as a first or second order reaction.

These results and the fact that examination of Van Petten's protocol books showed that he had left both arterial and venous samples at room temperature for 30 minutes or longer before the plasma and cells were separated, suggested that the low extraction ratios of PAH he reported were probably

due to passage of PAH from the red blood cells into the plasma in the venous samples. The average extraction ratio reported here is 0.772. This was calculated from all the immediate values. It is much lower than the figure of 0.90 quoted by Smith (1951 p 160), but is close to the figures reported by Asheim et al. 1958. Asheim's values ranged from 0.70 - 0.98 with an average of 0.82. The lower value, obtained in our study, may have been due to the 3-4 minute delay incurred by withdrawal time, mixing and dividing of the samples among the treatment tubes.

Various maneuvers, such as clamping the left renal outflow for periods of up to ten minutes, rapid and slow haemorrhages of up to 20% of the animal's estimated blood volume and stimulation of the sciatic and splanchnic nerves at varying frequencies and voltages were tried to determine whether a low extraction ratio could be produced, since installation of the meter alone did not produce very low extraction ratios. These maneuvers may also have contributed to the low average E_{PAH}. However, they did not appear to have the marked effects that Van Petten reported, since the lowest extraction ratio we calculated using the "immediate" values was 0.723.

Part II - India Ink Injection Studies

    (a) Methods

        Injection studies were done on seven dogs after the meter had been inserted. The method was similar to that used by Hoff et al. (1951) in their studies on cats. The kidneys were carefully freed from the dorsal body wall by blunt dissection and coarse strings were passed around between the kidney and the body wall and tied loosely. The carotid artery was isolated and a long catheter was inserted and passed down into the aorta. At this point the flowrate of the meter was checked and if no great change (less than 10%) had occurred in measured flow, about 200 ml. of a solution of India ink diluted half and half with distilled water were injected into the carotid catheter at a pressure slightly higher than systolic blood pressure. This pressure was maintained with a sphygmomanometer. After the perfusion was completed, the loose ties were quickly tightened and secured and the kidneys were removed and photographed. Renal clearance studies were done before and after the insertion of the meter. The studies completed just before the ink injection were taken as being representative of the state of the kidneys during the actual perfusion itself. It was assumed that neither the positioning of the coarse ties nor the India ink affected the intrarenal blood distribution pattern. All the dogs used were given mannitol to produce an osmotic diuresis. This was administered in the constant infusion (see methods) and a priming injection

was given in most cases after the meter was in place.

(b) Results

The results are shown in Figs. 4-9. Functions are given as ratios of the average control values on the respective sides. Thus values greater than 1.00 denote an increase and values less than 1.00 a decrease in the function involved. Actual values are given in Appendix 2.

(c) Discussion

These results do not support the theory that the oliguria was due to a cortex to medulla shunt. Although Fig. 6 seems to show such a shunt, upon checking the functions, it can be seen that while the functions of the left side are lower than those on the right, the E_{PAH} is very high at 0.89. Furthermore, both sides appear to be "shunted" and should, accordingly, have very depressed function. The absolute values are well within the "normal" range.

Since the extreme functional effects reported by Van Petten after insertion of the meter were not clearly evidant in the first four animals, sciatic nerve stimulation was per- formed on 10/1/59 as well as meter insertion. This may have been responsible for the extraction ratio of 0.74, and the L/R urine ratio of 0.68. However, the mechanism of the effect was apparently not through a cortex to a medulla shunt.

The most consistant effect of meter insertion seemed to be less blood flow through the medullary region of the left kidney to which the meter was attached, as compared to the

Figure 5
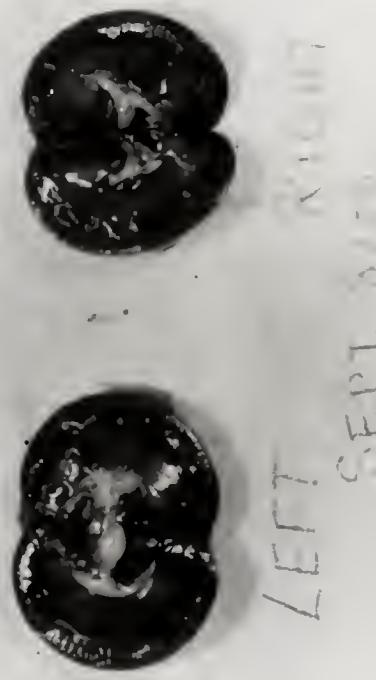
E_{PAH} .80
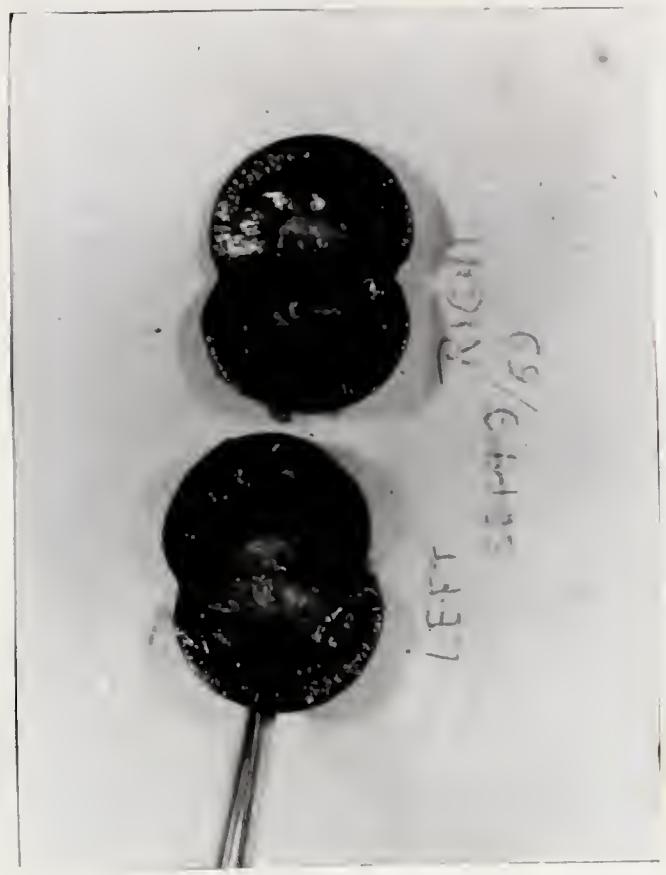
	V	GFR	ERBF	FF	Uosm
L	1.22	1.37	.47	2.93	.73
R	2.10	1.40	.61	2.24	.62

Figure 4

E_{PAH} .65

	V	GFR	ERBF	FF	Uosm
L	.55	.42	.68	.64	.92
R	.71	.57	.61	.94	.94

Figure 7

EPAH .75

	V	GFR	ERBF	FF	Uosm
L	1.18	.48	.77	.56	1.03
R	1.23	.45	.55	.80	1.09
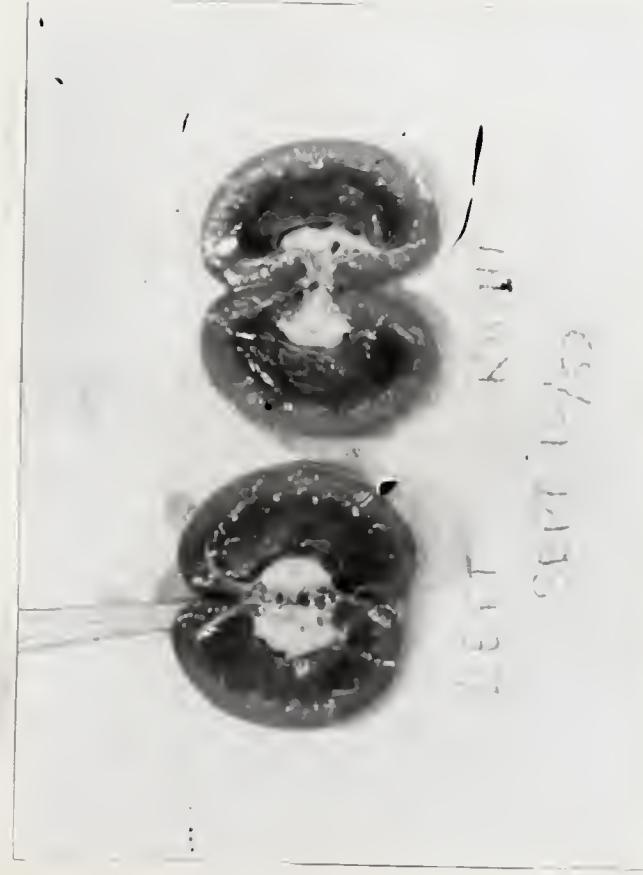
Figure 6

EPAH .89

	V	GFR	ERBF	FF	Uosm
L	4.08	.88	1.29	.68	.51
R	4.72	1.37	1.65	.78	.57

Figure 9

Anaesthetized

| V | L/R after | 1.26 |
| Uosm | L/R after | 0.96 |
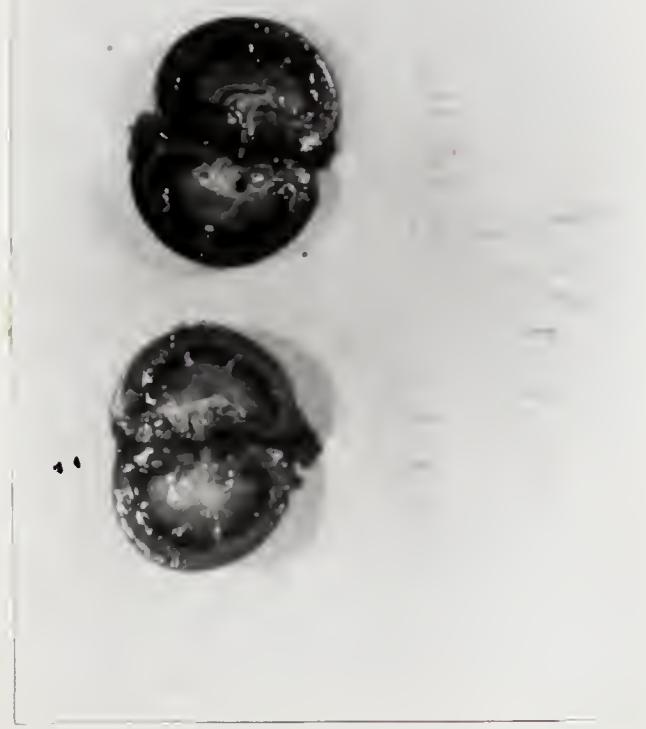
Figure 8

Stimulated after meter
EPAH .74

	V	
L	2.17	
R	2.57	
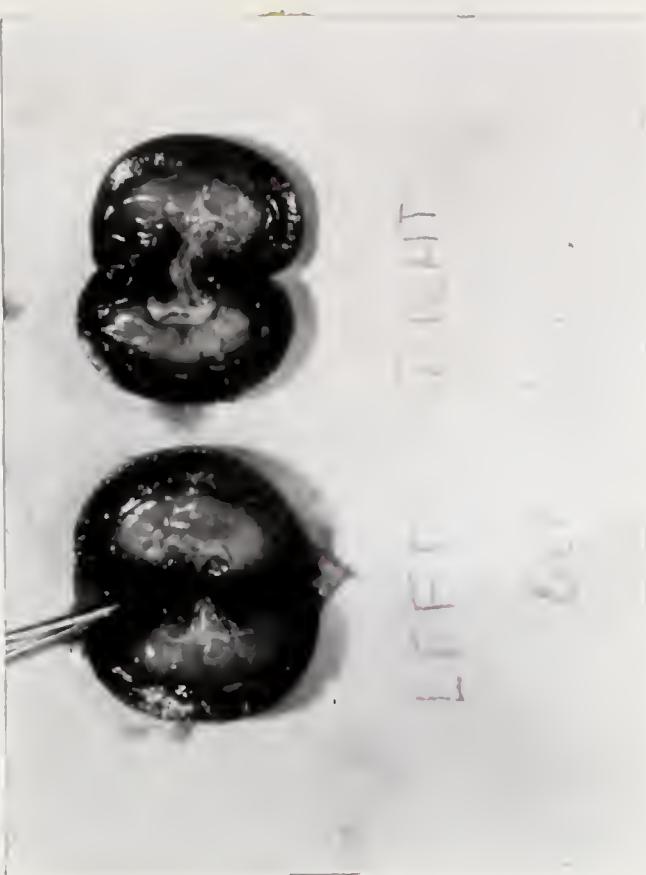
right kidney. This was indicated by less dense staining of the medullary region and is particularly evident in Figs. 5 and 7. Technical problems of reproduction do not show it clearly in the others. Fig. 9 shows the cross-section of anaesthetized kidneys. Both have pale medullas and dark cortices. This animal was not injected with India ink and no difference between the two kidneys was discernable. Two other sets of kidneys which were not photographed, but had unilateral oliguria showed a lighter medulla on the meter side. The meter was inserted into the left renal vein in one dog and into the right renal vein in the other.

Part II - The Role of the Concentrating Mechanism and Other Factors in Producing Oliguria

1.   General Results and Discussion

The average values of the various functions for each dog for each treatment period can be found in Appendix V. Each of the randomized treatment series was repeated three times and an incomplete randomization (dog #4) was included in the later discussion. Close examination shows several important differences between these results and those reported by Van Petten.

Van Petten reported that installation of the flowmeter into the left renal vein resulted in a decrease in the extraction ratio of PAH, the true filtration fraction and the GFR with little or no change in the renal blood flow. In

Table 5 - Extraction Ratio of PAH Changes

Treatment	C	M	A	P
Dog #				
1	--	.885	.815	.878
2	--	.771	.754	.822
3	--	.769	.771	.763
4	.812	.847	--	.862
5	.795	.795	.830	.852
6	--	.797	.804	.827
7	--	.871	.852	.835
8	.848	.879	.890	.904
9	--	.807	--	.856
10	,661	.789	.727	.785
$\bar{x}$	.779	.821	.805	.838

Table 6 - True Filtration Fraction Changes

Treatment	C	M	A	P
Dog #				
1	--	.24	.15	.21
2	--	.40	.44	.43
3	--	.26	.24	.25
4	.30	.30	--	.32
5	.21	.27	.30	.32
6	--	.31	.31	.28
7	--	.32	.21	.21
8	.35	.40	.36	.42
9	--	.27	--	.28
10	.18	.23	.18	.20
$\bar{x}$	.26	.30	.27	.29

C = control period   M = meter installation

A = kidneys anaesthetized   P = increased venous pressure

this series none of the treatments appeared to have any appreciable effect on either the extraction of PAH or the true filtration fraction as tables 5 and 6 show.

The venous samples necessary for these calculations were very difficult to obtain during the control periods because of technical difficulties. For that reason, there are only four control values to compare with the other values. Variance analysis could not be used because of the missing values. However, it appears to be a reasonably safe assumption that none of the treatments produced significantly different changes.

The higher extraction ratios were expected because of factors mentioned in Part I and the low TFF reported by Van Petten can probably be explained on the same basis. The TRPF values he calculated and used to determine TFF were presumably too high because of the low extraction ratios. Recalculating some of his data using the extraction ratio of 0.80 instead of 0.65 as he used gives a TFF of about 0.30 instead of 0.15 as he reported.

In view of the fact that his calculations of TRBF were undoubtedly high, it seems odd that the relationship between MRBF and TRBF differs in the two sets of experiments. Van Petten reports no significant difference between the MRBF and TRBF measurements on the basis of the "t" test, despite the fact that the average values for the MRBF were always higher than the corresponding values for the TRBF. In this

study variance analysis showed the average value of 70.2 ml/min-
ute for MRBF was significantly lower than the average value
of 76.1 ml/minute for TRBF. Recalculating Van Petten's data,
using variance analysis, indicates that the average value of
8.7 ml/minute/Kg for the MRBF is significantly higher than
the average value of 7.4 ml/minute/Kg for TRBF which is in
itself too high. The reason for this discrepancy is not
obvious.

The GFR decreased after meter installation as in
Van Petten's experiments. However, in most cases the decrease
was not as great. The changes in TRBF tended to follow
changes in the GFR which would be expected from the small
changes which occurred in the TFF.

Table 7 - Renal Venous Pressure Changes

Treatment	C	M	A	P
Dog 1	9	19	29	34
2	10	26	28	33
3	9	27	29	34
4	11	30	--	33
5	8	20	21	28
6	10	19	23	26
7	6	25	21	32
8	7	18	5	25
9	12	--	10	--
10	7	14	6	17
$\bar{x}$	9	22	--	29

C = control   M = meter installation   A = local anaesthetic

P = venous pressure raised

Table 8 - Variance Analysis of Changes in Urine Volume

Treatment	C		M		A		P		Sum of Replicates
Side	L	R	L	R	L	R	L	R	
Dog #									
1	1.8	2.0	1.3	1.7	2.0	3.3	1.3	3.8	17.2
2	2.4	2.5	3.8	4.4	2.8	6.2	2.6	4.7	29.4
3	7.0	7.3	8.7	12.3	6.7	8.4	7.8	11.0	69.2
5	4.8	4.5	4.2	5.0	6.1	8.2	4.2	7.5	44.5
6	4.5	5.1	4.3	4.4	8.5	9.4	4.6	10.4	51.2
7	7.2	8.0	6.7	4.8	4.9	4.8	4.4	4.2	45.0
8	4.1	3.6	4.1	2.8	4.4	3.6	2.4	1.8	26.8
9	1.9	3.4	5.4	6.7	3.2	4.5	5.7	6.8	37.6
10	7.3	6.7	5.6	6.8	7.9	7.2	3.9	5.9	51.3
Sum of Interaction	41.0	43.1	44.1	48.9	46.5	55.6	36.9	56.1	

Sum of Sides   L - 168.5   R - 203.7

Sum of Treatment   C - 84.1   M - 93.0   A - 102.1   P - 93.0

Total   372.2

S of V	DF	SS	MS	F	1%	5%
Replicates	8	243.33				
Sides	1	17.21	17.21	7.55	7.12	
Treatment	3	9.00	3.00	1.31		4.16
Interaction (SxT)	3	9.39	3.13	1.37		4.16
Error	56	127.70	2.28			
Total	71					

Note:-   actual randomizations were as follows:
Dogs 1-3 CMPA, Dogs 5-7 CMAP, Dogs 8-10 CAMP

C = control period   M = meter installation

P = increased venous pressure   A = kidneys anaesthetized

Renal venous pressure, measured from the tip of a catheter in the renal vein, was much greater after meter installation than the values of 10-15 mm Hg reported by Van Petten. Examination of Table 7 shows that the pressure rose from an average of 9 mm Hg (range 6-12) to an average of 22 mm Hg (range 14-30) after installation of the meter and rose further to an average of 29 mm Hg (range 17-34) after partial occlusion of the outflow catheter. In 1949 Blake et al. and Selkurt et al. published their findings of the effects of increasing the renal venous pressure to these magnitudes. The relation of their results to the results of this investigation are discussed later.

Variance analysis of the effects of the treatments on urine production showed odd results (Table 8). The analysis indicates that the left side on the average produced significantly less urine than the right side. However, the various treatments did not produce any significant change in urine production, as the small F value for treatments shows, and did not have a significant selective action on the left side as would be expected from meter installation and raising the venous pressure. The latter effect is demonstrated by a low F value for the interaction of sides and treatments (SXT).

These results can be explained on two basis. First, there is a general tendency for urine production to increase on both sides during the course of an experiment and secondly, the same treatments appear to produce different effects in

different animals. For example, with Dog #7 installation
of the meter decreased urine volume on the right side more
than on the left, the anaesthetic had more apparent effect on
the left side than on the right, and increasing the renal
venous pressure on the left side only affected both sides
approximately the same. This is almost exactly opposite to
Dog #5. Other examples can be seen on close examination.

The bilateral increase in V is probably due to an
increased osmotic diuretic effect from increased plasma levels
of urea. Plasma urea levels were not measured, but increases
in urine output parallel increases in plasma osmolarity (Posm)
which were measured. No attempt was made to control plasma
levels of urea since urea does not affect the production of
ADH (Verney 1948) and is non-toxic even at high plasma levels.
The parallel changes in the percentage of water and solute
reabsorbed during the course of an experiment and in urine
output and osmolar clearance also suggests that the increase
in urine output was due to increased osmotic effects (see
appendix).

It was decided to compare the L/R ratios of urine
production instead of using the raw data. When ratios are
compared in this way, it is assumed that factors which act
bilaterally do not change the ratios of the functions even
though the absolute values may change. It is also assumed
that any treatment applied unilaterally affects only the
treated side. This method was used by Blake et al. (1949) and

Table 9 - <u>Variance Analysis of Changes in L/R V Ratios</u>

Treatment	C	M	A	P	Sum of Replicates
Dog #					
1	.90	.76	.61	.34	2.61
2	.96	.86	.45	.55	2.82
3	.96	.71	.80	.71	3.18
5	1.07	.84	.74	.56	3.21
6	.88	.98	.90	.44	3.20
7	.90	1.40	1.02	.85	4.17
8	1.14	1.46	1.22	1.33	5.15
9	.56	.81	.71	.84	2.92
10	1.09	.82	1.10	.66	3.67
Sum of Treatments	8.46	8.64	7.55	6.28	
Total					30.93
$\bar{x}$	.94	.96	.84	.70	

S of V	DF	SS	MS	F	5%
Replicates	8	1.2579			
Treatments	3	.3885	.1295	4.15	3.01
Error	24	.7479	.03116		
Total	35				

$$LSD = \sqrt{\frac{.03116 \times 2}{9}} \times 2.06 = 0.17$$

Note:- actual randomizations were as follows:
Dogs 1-3 CMPA, Dogs 5-7 CMAP, Dogs 8-10 CAMP

C = control period   M = meter installation

P = Increased venous pressure   A = kidneys anaesthetized

has been used extensively by Selkurt (Bradley 1951). Comparing ratios removed all variability in this data produced by the general trend of increased urine production on both sides and by the general tendency of the left side to be lower than the right side and left only the variability due to replicates and treatments.

Statistical analysis of the L/R ratios of urine production is shown in Table 9. The LSD of 0.17 shows that raising the venous pressure is mainly responsible for this significant effect. The average value for the ratio produced by this treatment, 0.70, is significantly lower than the averages of 0.94 and 0.96 for the control and meter installation respectively. The average ratio of 0.84 for the effect of anaesthetic does not differ significantly from the other averages.

The different effects produced by the same treatment in different animals are more obvious in this presentation of the data. Because it became apparent that the treatments were in fact producing different effects in different animals, it was decided to compare changes in the L/R V ratios with changes in the ratios of other functions measured simultaneously for both kidneys. Particular notice was made of changes in the L/R ratios of GFR and Uosm. All the changes in the L/R V ratios could be associated with four types of change in the GFR and Uosm ratios. These four types of change can be

described as follows:

(1) Changes in the GFR L/R ratios only, in the same direction as the changes in the V ratio.

(2) Changes in the Uosm L/R ratios only, in the opposite direction to the change in the V ratio.

(3) Changes in the GFR ratios in the same direction as changes in the V ratio with changes in the Uosm ratios in the opposite direction. These are described as complementary changes.

(4) Changes in the GFR ratios in the same direction as changes in the V ratio with changes in the Uosm ratios also in the same direction as the V ratio changes. These are described as non-complementary changes.
Complementary changes tend to augment each others effects whereas non-complementary changes tend to cancel each others effects. Because none of these four classifications of changes appears to be exclusively associated with any particular treatment or animal, it is difficult to assign any definite mechanism to any of the changes. The "M" and "P" treatments are associated with increases in the left renal venous pressure. In general the "A" treatment produced little change in the venous pressure. All three treatments produced changes in the arterial pressure, although not in every animal. Such a change should have a bilateral effect unless there is an interaction between arterial pressure and renal venous pressure.

In the following section an attempt has been made to

explain the mechanism of these four changes. Unfortunately the explanations incorporate some speculation because the experiments were not designed to demonstrate these effects. Such an experiment would be difficult to design because of the necessity of having very accurate control over the renal arterial blood pressure and the renal venous pressure, without damaging the nerve supply to the kidney or upsetting its normal function.

2.   Discussion of the Mechanisms of Changes in Urine Production

In the following sections each mechanism is discussed separately with each of the separate treatments which produced the change listed. Each treatment is designated by the dog number and a treatment symbol. Thus, "1M" means the effect of meter installation on Dog #1. Changes of less than 0.03 in a ratio have been arbitrarily classed as no change.

The changes in ERPF ratios have been included to show the relationship between this parameter and the other measurements. It can be seen that in general they tend to follow the changes in the L/R V ratios except in Uosm changes where this fact is discussed. The absolute changes in some of the functions for the left side are also included. It will be noticed that in general there are only small changes in the TFF and the changes in E_{PAH} do not appear to be related to the changes in the other functions. The relation of changes in arterial and left renal venous pressure are discussed in

the appropriate place in each of the discussions of the mechanisms.

(a) GFR Changes

Table 10 - GFR Changes

	V decreased				V increased			
Treatment	1M	1P	5A	7A	6M	8M	9P	8A
Ratio Changes								
V	-.14	-.42	-.10	-.38	.10	.24	.03	.08
GFR	-.12	-.19	-.14	-.09	.07	.09	.04	.03
Uosm	-.01	.02	.01	.01	-.01	.00	.00	.02
ERPF	-.46	-.32	-.03	-.16	.02	.15	.06	.11
Absolute Changes								
LE_{PAH}	--	-.007	.035	-.019	--	-.011	.049	.042
AP	-39	-2	-11	-6	-25	-7	-6	-4
LVP	10	15	1	4	9	12	--	-1.5
LTFF	--	-.03	.03	-.11	--	.04	.01	.01

The decreases in GFR ratio which occurred in 1M and 1P can be attributed to the unilateral increase in venous pressure since the arterial pressure changes would have a bilateral effect. There is a possibility that there may have been an interaction between the arterial pressure decrease and the venous pressure increase in 1M, but this cannot be proved.

The reason for the decreases in the GFR ratios in 7A and 5A are not so obvious. Theoretically anaesthetizing both kidneys should not change the ratios because this is a bilateral treatment. However, if one of the kidneys has been

given a unilateral treatment before the anaesthetic admin-
istration the effect on both kidneys need not be proportional
and the ratio will change. The treatment applied to the left
kidney before both kidneys were anaesthetized was installation
of the meter in both of these dogs. If the effects of meter
installation had been only reflex in nature, the anaesthetic
would have affected the left functions more than the right
and the GFR ratio would have increased. The decrease suggests
that the effect of the meter installation was mainly mechanical
since the right GFR was relatively higher after bilateral
anaesthesia than the left GFR. The large decrease in true
filtration fraction which occurred in 7A on the left side
suggests that some of the effects of meter installation, at
least in this case, were reflex as well as mechanical. The
decrease in TFF occurred because the TRPF was larger in com-
parison to the GFR than it was during the "M" treatment
period. This is the condition which would be expected if the
"M" treatment had reduced the TRBF by a reflex mechanism.

Two of the increases in GFR ratio (6M and 8M) and
probably a third (9P) occurred in the face of substantial
increases in renal venous pressure. It is possible that this
could be due to over compensation of the autoregulatory
mechanism, which was changed by the increases in venous
pressure. Haddy et al. (1958) have suggested that increases
in renal venous pressure control the RBF and presumably the
GFR by both a mechanical passive mechanism and an active

"venous-arteriolar reflex". The latter mechanism if it is local would not be affected by anaesthetic and might explain why this mechanism appears to act in both anaesthetized (8M and 9P) and non-anaesthetized kidneys (6M). The increase in the GFR ratio in 8A, where the kidneys were anaesthetized after the control period, may have been produced by blocking of sympathetic activity initiated in the left kidney by positioning of the ties and catheterization of the gonadal vein before the control period. This would cause the left side to change relatively more than the right side as was explained previously.

(b) Uosm Changes

Table 11 - Uosm Changes

	V decreased			V increased	
Treatment	3M	4M	6A	3A	4P
Ratio Changes					
V	-.25	-.20	-.08	.13	.09
GFR	-.01	.01	-.02	-.01	-.01
Uosm	.07	.10	.03	-.05	-.10
ERPF	.05	.03	.01	-.09	-.09
Absolute Changes					
LE$_{PAH}$	--	.035	.007	.015	.008
AP	20	-6	-29	9	-4
LVP	18	19	- 4	3	-5
LTFF	--	.00	.00	.02	-.01

The changes in Uosm ratio can be explained by unilateral changes in the efficiency of the concentrating mechanism. It is possible that under some circumstances the division of outflow from the juxtamedullary glomeruli varies so that less blood flows through the vasa recta and more flows through the peritubular capillary network (Fig. 1). The mechanism of the effect is probably initiated by an increase in renal venous pressure, either mechanically by preferential partial occlusion of the medullary blood flow and/or by a reflex mechanism. The fact that this pattern occurred in only three dogs, and twice in two of these with different treatments (Table 11) suggests that anatomical characteristics may also be involved.

The reduction of flow through the vasa recta must only be to a small degree, insufficient to interfere with oxygen supply to the solute carrier enzymes in the counter-current mechanism, but enough to allow more complete equilibration to occur between the arms of the vasa recta by means of countercurrent exchange. Thus less solute would be removed from the medulla by vasa recta blood flow providing a greater solute pool for urine concentration. This would produce a smaller volume of more concentrated urine as occurred in 3M, 4M and 6A. With increased vasa recta flow the reverse could occur as in 3A and 4P. This hypothesis is supported by the concomitant increase in ERPF ratios which occurred when V ratios decreased and the decrease in these ratios when the V

ratios increased. Changes in ERPF reflect changes in flow
through the peritubular capillary network, the portion of the
kidney where much of the PAH is removed from the plasma.

The work of Blake et al. (1949) may be explained
by this hypothesis since they reported similar effects after
increasing renal venous pressure. They did not measure the
urine osmolality and attributed the decrease in V ratios
without a decrease in GFR ratios to mechanical effects rather
than humoral or central reflex mechanisms. Almost one third
of their original experiments (seven out of 22) were discarded
because of an absolute decrease of more than 25% in the GFR
during the experimental procedure. In addition to this, any
animal in which the L/R ratio of the functions measured in
the control period fell outside of the range 0.90-1.10 was
also rejected. This method of selection may have biased
their results. The five treatments discussed here meet the
first criterion but Dog #3 does not meet the second, so the
selection method cannot be the sole cause of Blake's results.
Possibly he used a more uniform type of dog.

(c) Complementary Changes in GFR and Uosm

The controlling factor in this mechanism is probably
the GFR. (See Table 12). Examination of equation (2) in the
literature survey shows that as "u", the velocity factor,
decreases, the maximum concentration that can be produced by
the countercurrent multiplication system increases. In

addition to this, equilibration between the collecting ducts
and the medullary interstitium is more complete at low flow
velocities.

Table 12 - Complementary Changes in GFR and Uosm

Treatment	V decreased					V increased		
	10M	2P	6P	10P	2A	7M	1A	9A
Ratio Changes								
V	-.28	-.31	-.46	-.16	-.10	.50	.27	.15
GFR	-.07	-.09	-.29	-.16	-.11	.17	.30	.08
Uosm	.19	.08	.14	.07	.03	-.23	-.03	-.14
ERPF	-.42	-.24	-.32	.02	-.09	-.04	.40	.11
Absolute Changes								
LE_{PAH}	.062	.051	.023	-.004	-.068	--	-.063	--
AP	-33	4	1	-4	-13	-15	-21	23
LVP	8	7	3	3	- 5	19	- 5	-2
LTFF	.05	.03	-.03	.01	--	-.06	--	--

An increase in GFR which increases "u" produces the opposite
effect. Results similar to these were described by Levinsky
et al. (1959a). They found that maximally concentrating
kidneys would produce more concentrated urine if the GFR was
reduced by partial constriction of the renal artery. This
effect occurred only with reductions down to an average of
70% of control. They attributed this phenomenon to decreased
"solute load" to the collecting tubules. Presumably this
refers to the more complete equilibration which can occur
between the collecting ducts and the medullary interstitium.

They make no mention of increased efficiency of the counter-current concentrating mechanism.

The factor which changes the GFR to produce these changes is not clear. It is probably mechanical and not reflex because all the treatments except 2P and 7M were performed on anaesthetized kidneys. It is probably due to increases in renal venous pressure which is the main unilateral effect, although an interaction between arterial and venous pressure changes may be responsible. An anatomical factor may also be involved since two separate treatments worked in the same manner in dogs #2 and #10.

(d)   Non-Complementary Changes in GFR and Uosm

The primary factor in this mechanism also appears to be the GFR. (See Table 13.) In each case the GFR decreased as well as the concentration of the urine. In two cases the decrease in urine concentration appeared to compensate for the decrease in GFR (3P and 10A) and in one case (9M) there appeared to be over compensation and the V ratio actually increased.

Treatment 8P does not at first appear to fit this trend, however, examination of the absolute values (Appendix V) shows that the GFR fell considerably on both sides while the urine concentration fell only on the left or treated side and actually increased on the right side. This returned conditions to what they had been during the control period, which suggests

that the anaesthesia may have been leaving the right side and it no longer was a valid control. In any case the mechanism is obviously one of non-complementary change in GFR and Uosm.

Table 13 - Non-Complementary Changes in GFR and Uosm

	V decreased					V same		V increased
Treatment	5P	7P	8P	2M	5M	3P	10A	9M.
Ratio Changes								
V	-.18	-.17	-.13	-.10	-.23	.00	.01	.10
GFR	-.13	-.15	-.02	-.41	-.18	-.09	-.06	-.11
Uosm	-.06	-.03	-.10	-.05	-.04	-.08	-.03	-.09
ERPF	-.22	-.12	-.07	-.02	-.17	-.06	.01	-.15
Absolute Changes								
LE$_{PAH}$	.022	-.017	.025	--	.00	-.006	.006	--
AP	-8	-3	1	-13	-23	4	0	4
LVP	7	11	7	16	12	7	-1	--
LTFF	.02	.00	.02	--	.06	-.01	.00	--

Levinsky et al. (1959a) reported results like this after reductions in GFR to an average of less than 70% of control. In some animals they reported that the urine became more dilute after only a 10% reduction. They explained the phenomenon by suggesting that the reduced GFR did not supply adequate amounts of solute to the solute concentrating mechanism in the thick ascending loop and, therefore, its efficiency was impaired. However, if the GFR is reduced,

there will be less urine to be concentrated and, therefore, the decreased efficiency should not matter.

Another explanation which seems more reasonable is reduction of juxtamedullary blood flow to the extent that the oxygen supply to the enzyme systems of the thick ascending loop of Henle is interfered with. The juxtamedullary glomerular outflow is the sole blood supply to the medulla. Reduction of renal blood flow and GFR by increasing arterial occlusion would affect the juxtamedullary glomeruli last because of their close proximity to the arcuate artery. Thus the results of Levinsky et al. may be explained in this way. The initial constriction of the renal artery reduced only cortical glomerular GFR and had no effect on the juxtamedullary function. Therefore, the concentrating mechanism efficiency was, if anything, increased and the amount of urine presented to it was smaller and a higher concentration was accomplished. As the amount of constriction increased the juxtamedullary glomerular blood flow and GFR were reduced. At some point the solute enzyme carrier systems in the thick ascending loop began to fail due to hypoxia and the concentrating efficiency was reduced. Therefore, more dilute urine was produced.

In the experiments described here the factor decreasing juxtamedullary blood flow and GFR such that anoxia developed in the thick ascending loop was probably increased renal venous pressure occluding vasa recta flow or

its interaction with decreased arterial pressure. Since the effect occurred in both anaesthetized and non-anaesthetized kidneys it appears to be mechanical in nature. In 10A where the changes in each kidney should have been proportional because of no previous unilateral treatment the explanation used for 8A (p 70) may be invoked.

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSIONS

1.   General Discussion

The effects of meter installation on urine production were not so clearcut as those reported by Van Petten. The unilateral oliguria observed during this work was not so severe and in some cases it did not occur at all. This suggests that the experimental conditions encountered by Van Petten were not duplicated here. There is no obvious reason why they should not have been duplicated, but some factors may partially explain the differences.

One possibility is that Van Petten's experimental animals may have been in the primary stages of shock. He had trouble in maintaining adequate levels of arterial blood pressure after meter installation and discarded several experiments for this reason. His experimental animals may, therefore, have responded in a different way to meter installation than the animals used in this study, all of which were in relatively good condition throughout the experiment. In no cases did the arterial blood pressure fall below 100 mm Hg in this study and there was little evidence of "shock" waves in any of the blood pressure tracings. Another difference was the administration of ADH and urea to the animals in the last series to produce a hypertonic diuresis. However, in the preliminary series of experiments these two substances were not administered to the experimental animals and the unilateral oliguria in these experiments was also minimal or absent.

In 1949 two different groups of investigators,
Blake et al. and Selkurt et al., published results of the
effects of increasing renal venous pressure on various renal
functions. As has already been mentioned, Blake's results
may fit into the second category of changes since he reported
a decrease in L/R V ratios in response to increases in renal
venous pressure with no change in the L/R GFR ratios. Selkurt
et al. measured only the left renal functions, the kidney in
which the venous pressure was raised. They found that renal
functions tended to decrease during their experiments and
reported that increasing the renal venous pressure to above
an average of 22.4 mm Hg produced a decrease in GFR and TRBF
significantly lower than the decrease predicted from a "trend"
determined by using control animals. They found that the
filtration fraction (creatinine clearance/PAH clearance) did
not change. Urine volumes were not reported. The results of
both groups have, in effect, been duplicated here. However,
because they were interested in one specific application of
this information, namely, the relation of renal venous pressure
to the edema of cardiac failure, they may have selected
methods and results which tended to support preconceived
hypotheses.

The results reported here show that changes in GFR
can be implicated as the major factor affecting urine pro-
duction in 24 out of 29 cases. In the other five cases changes
in the concentrating mechanism appear to be responsible. In

sixteen cases changes in urine concentration are associated with the changes in GFR. An explanation of the relation between these two parameters is offered, showing how, when the juxtamedullary blood flow is seriously curtailed, a decrease in GFR may occur concomitantly with the production of more dilute urine eight out of sixteen times, and how, when only the cortical blood flow is reduced, a decrease in GFR can occur concomitantly with an increase in urine concentration eight out of sixteen times.

Unfortunately the hypothesis offered only explains the results obtained but the results do not prove the hypothesis. A series of experiments designed to show only the effects of increased renal venous pressure, more carefully controlled than these experiments and without the complicating variable of meter installation, anaesthetizing the renal nerves, or decreases in arterial blood pressure, would possibly produce predictable effects.

2. Conclusions

1. Installation of a bubble flowmeter into the left renal vein of dogs produces unpredictable unilateral changes in urine production. In the majority of cases the L/R urine ratios decrease but some increase or remain the same.

2. The main factor involved in these changes in urine volume appears to be unilateral changes in GFR.

3. The changes in GFR are probably produced by large

increases in renal venous pressure produced by increased
resistance to outflow occurring in the external flow circuit,
rather than from an intrarenal diversion of blood from
functional to non-functional tissue as had been previously
postulated. Further increasing the renal venous pressure
after meter installation, by partial occlusion of the outflow,
reduced the L/R urine volume ratio significantly (p less than
0.05).

4. The mechanism of the effect of the meter installation
may be partly reflex in nature, since pharmacological denerv-
ation of the kidneys with a local anaesthetic modifies the
response. However, it does not relieve it completely or
prevent it and the major mechanism is probably of a mechanical
nature.

5. In some cases the changes in urine volume appear to
be associated with increased or decreased efficiency of the
concentrating mechanism. A possible explanation involving
countercurrent principles and the anatomy of the juxtamedullary
circulation is offered.

6. Because these major unilateral effects of installation
of the meter cannot be predicted, and their precise mechanism
is obscure, it appears that modifications of the meter to
reduce its resistance to flow will be necessary before it can
be used to investigate changes in renal blood flow occurring
in response to hypervolemia.

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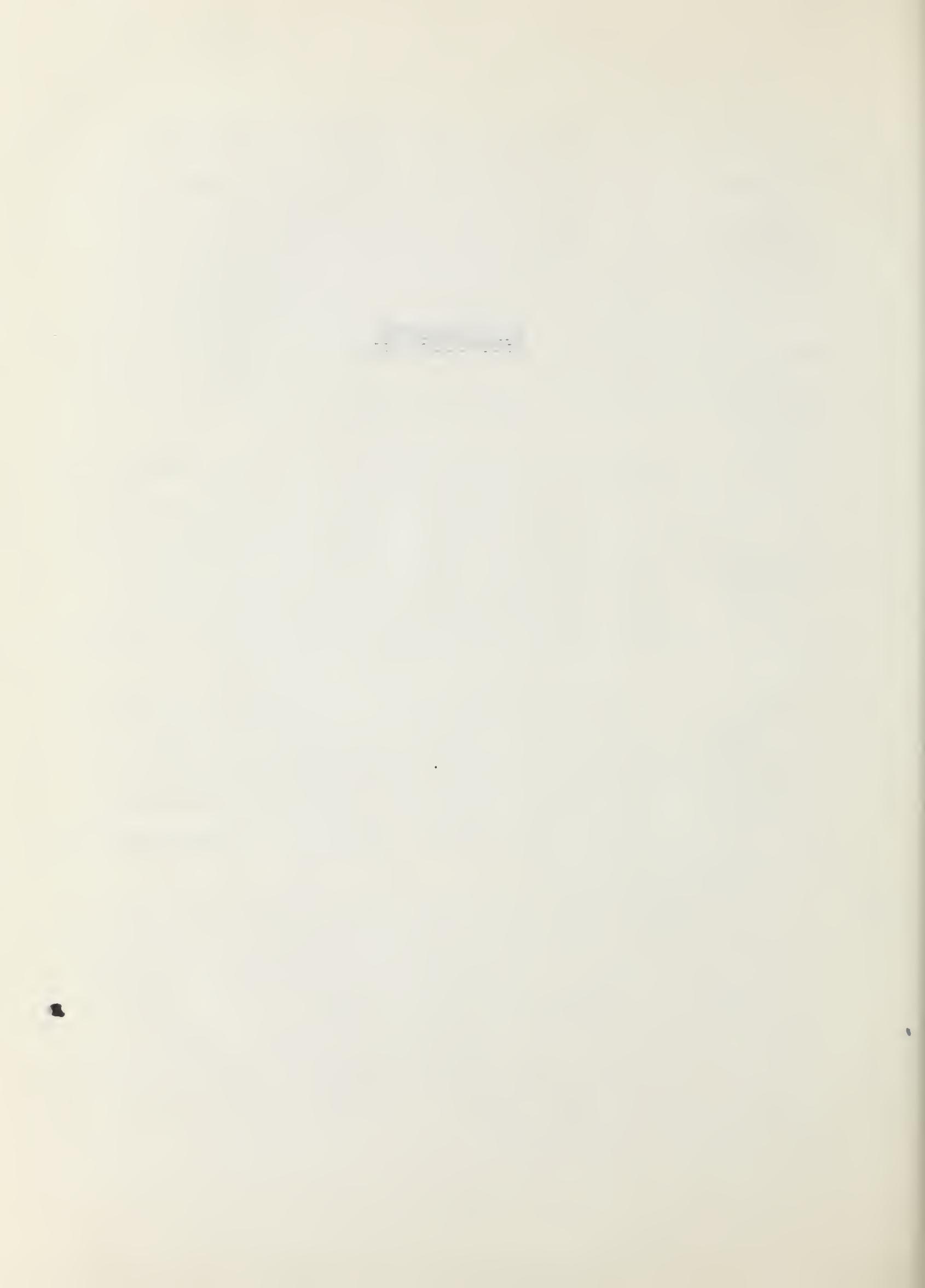
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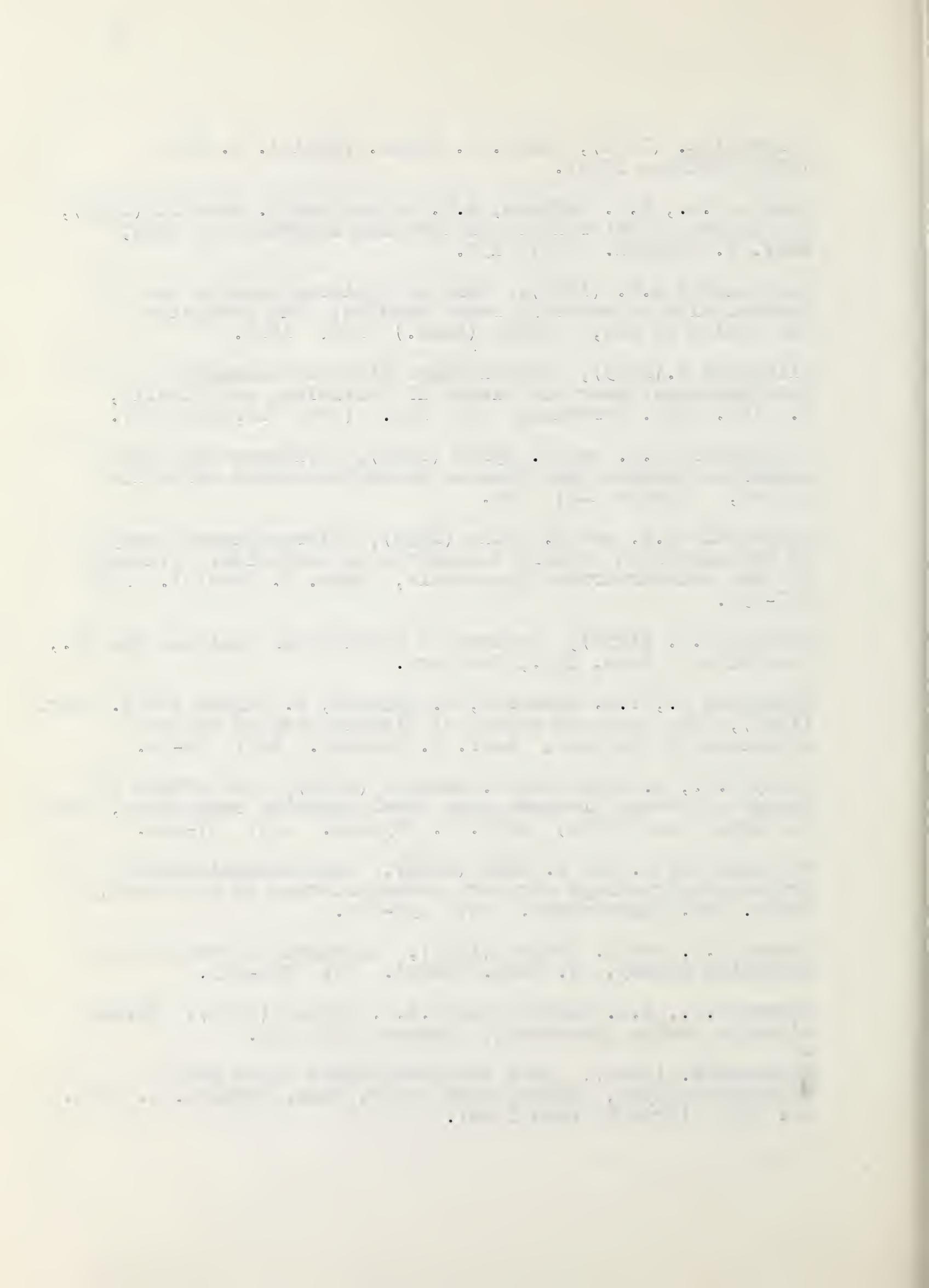
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Addendum

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APPENDICES

Abbreviation Guide

ERPF	Effective Renal plasma flow ml/min.
ERBF	Effective Renal blood flow ml/min.
TRPF	True or total renal plasma flow ml/min/
TRBF	True or total renal blood flow ml/min/
E_{PAH}	Extraction ratio of PAH
MRBF	Metered renal blood flow ml/min.
AP	Arterial Pressure mm Hg
RVP	Renal Venous Pressure mm Hg
GFR	Glomerular Filtration rate ml/min.
TFF	True filtration fraction
V	Urine volume ml/10 min.
%WR	% water reabsorbed
Posm	Plasma osmolality milliosmoles/Kg H_2O
Uosm	Urine osmolality milliosmoles/Kg H_2O
Uosm V	Solute output
% SR	% solute reabsorbed
Cosm	Osmolal clearance
C	Control period
M	Meter Installation
P	Increased venous pressure
A	Kidneys anaesthetized
S of V	Sources of Variance
DF	Degrees of Freedom
SS	Sum of differences squared
MS	Mean standard deviation squared or variance
FPD	Freezing point depression
CCM	Countercurrent multiplication
L/R	Left side over right side ratio
L	Left side
R	Right side

Appendix II

Dog #	9/8/59		9/9/59		9/10/59		9/11/59		10/1/59		10/5/59	
Function	B	A	B	A	B	A	B	A	B	A	B	A
LV	6.7	3.7	2.5	3.1	1.4	5.7	3.1	3.7	0.6	1.3	--	3.8
RV	6.3	4.5	3.8	8.0	1.4	6.6	3.1	3.8	0.7	1.8	--	3.0
Art. BP	122	97	137	127	133	133	131	111	--	--	--	--
L GFR	25.5	10.6	15.9	21.8	20.3	17.9	8.3	4.0	--	--	--	--
R GFR	26.7	14.5	26.3	36.9	15.3	21.0	8.9	4.0	--	--	--	--
L ERBF	134	90.7	176	82.5	118	152	72	55.3	--	--	--	--
L MRBF	--	75.3	--	80.2	--	122	--	45.2	--	--	--	--
R ERBF	137	83.5	219	135	94.5	156	87.3	48	--	--	--	--
L E_{PAH}	--	.65	--	.80	--	.89	--	.75	--	.74	--	--
L TFF	.34	.22	.19	.56	.31	.21	.20	.11	--	--	--	--
R TFF	.35	.32	.26	.58	.29	.22	.17	.14	--	--	--	--
L Uosm	647	595	710	520	1300	667	600	618	--	--	--	820
R Uosm	660	620	655	406	1150	660	575	626	--	--	--	857

Dog #	8/18/59		8/25/59		10/15/59		10/17/59		10/22/59	
Function	B	A	B	A	B	A	B	A	B	A
LV	3.8	4.5	2.3	8.1	2.1	1.2	4.4	8.1	12.1	6.6
RV	3.5	4.5	2.4	3.3	2.3	2.1	4.7	12.3	12.2	9.1
L Uosm	855	710	483	370	852	550	832	378	947	880
R Uosm	940	847	505	385	573	405	705	332	900	685

B - before meter   A - after meter

Appendix III

Urine Creatinine Standard Curve
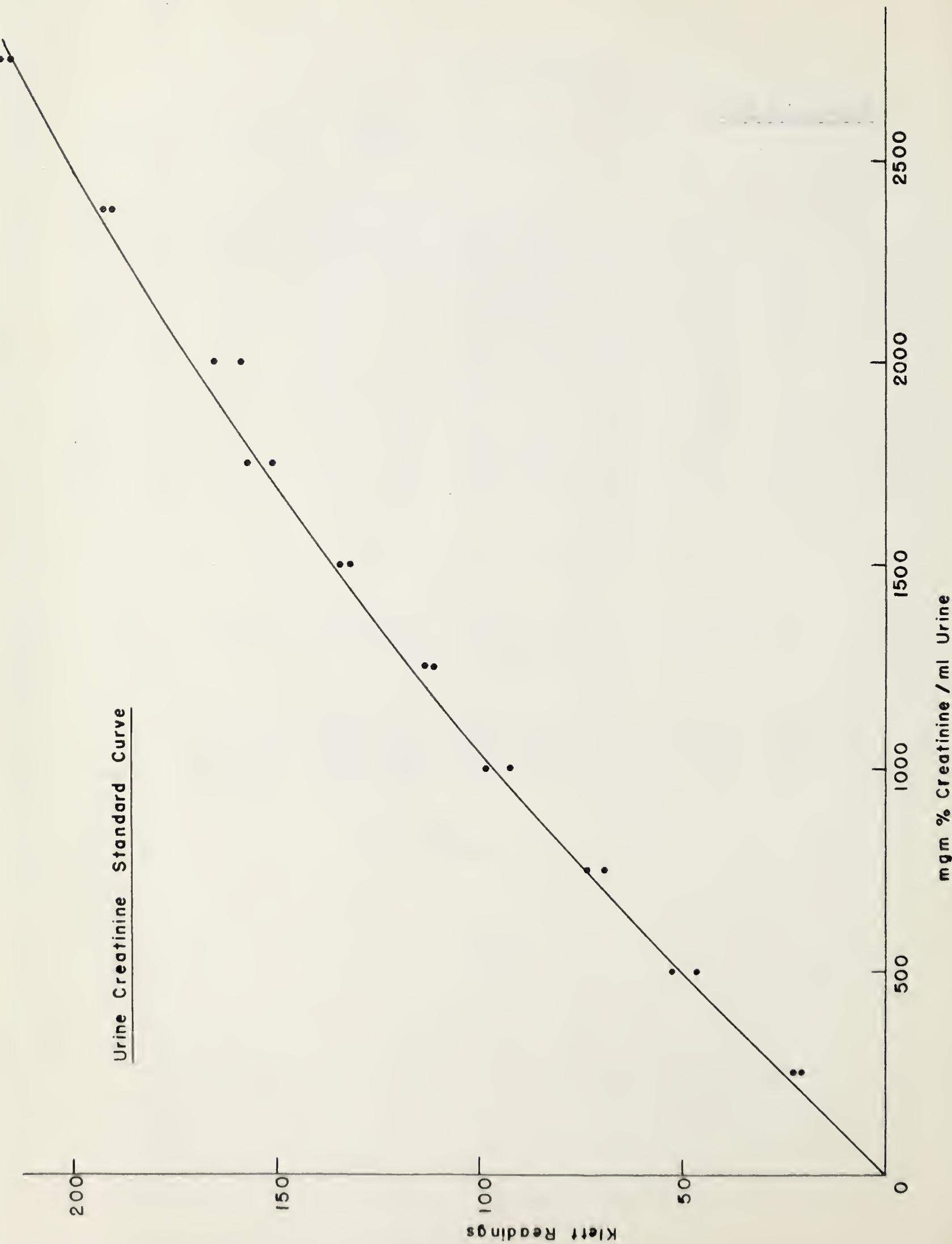
Raw Data for Urine Creatinine Standard Curve (1/2/60)

Klett Readings		Mgm % Creatinine/ml urine
0	0	0
20.5	22.5	250
52.5	46.5	500
69.5	73.5	750
98.5	92.5	1000
113.5	111.5	1250
132.5	134.5	1500
157.5	151.5	1750
166.5	159.5	2000
191.5	193.5	2375
217.5	218.5	2750
236.5	230.5	3125
255.5	251.5	3500
266.5	266.5	3875

Urine PAH Standard Curve
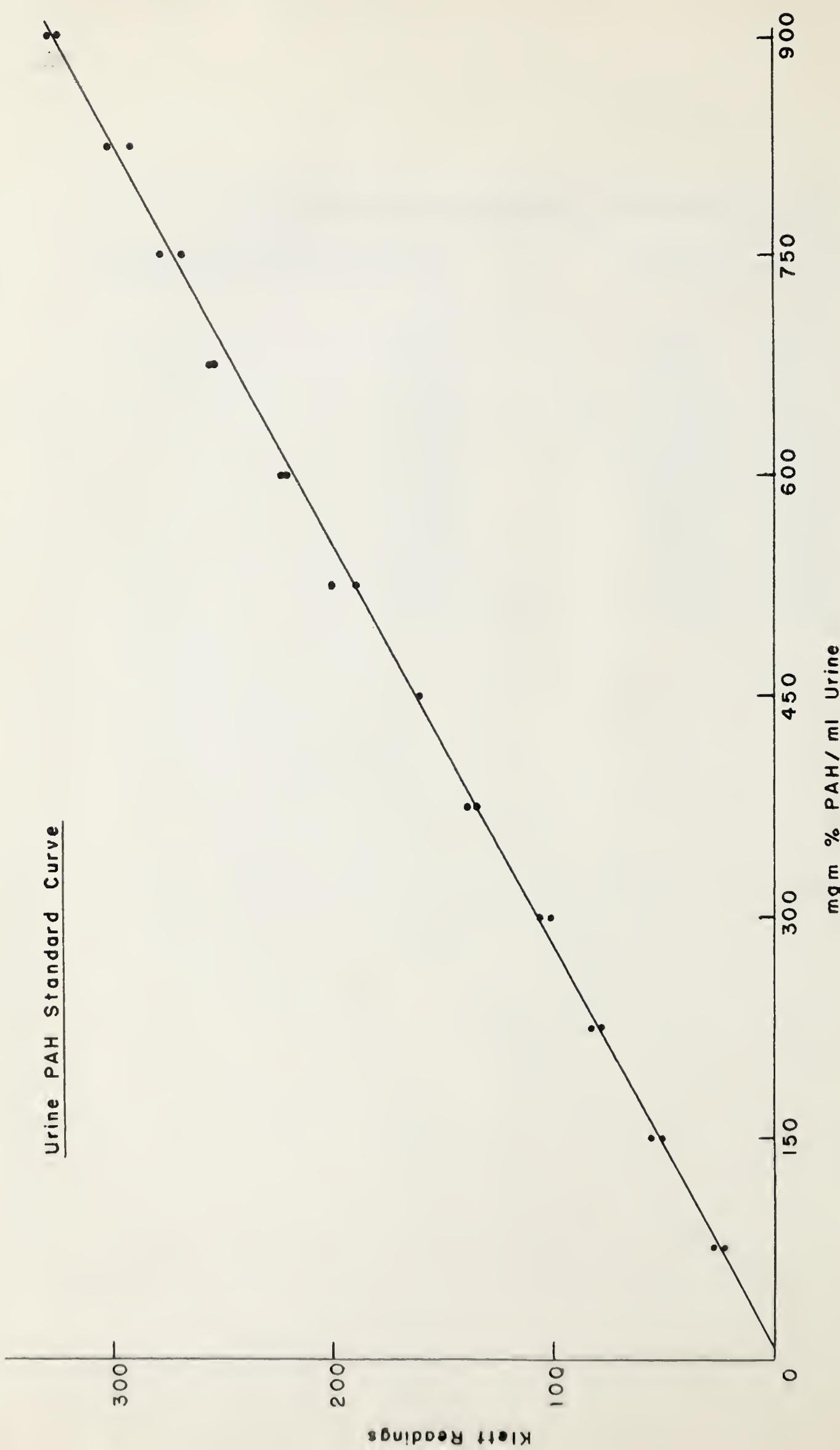
Raw Data for Urine PAH Standard Curve (1/2/60)

Klett Readings		Mgm % PAH/ml Urine
0	0	0
22.5	27.0	75
51.5	55.5	150
78.5	82.5	225
101.5	106	300
135	139	375
161.5	161.5	450
190	200	525
221	223	600
254	246	675
269	279	750
293	303	825
331	326	900
356	359	975
392	381	1050

Regression Values

$n = 30$

$byx = 2.7087$

$a = 6.7$

mgm % PAH/ml urine $= K.R. \times 2.7087 + 6.7$

ix

Plasma Creatinine Standard Curve

Klett Readings
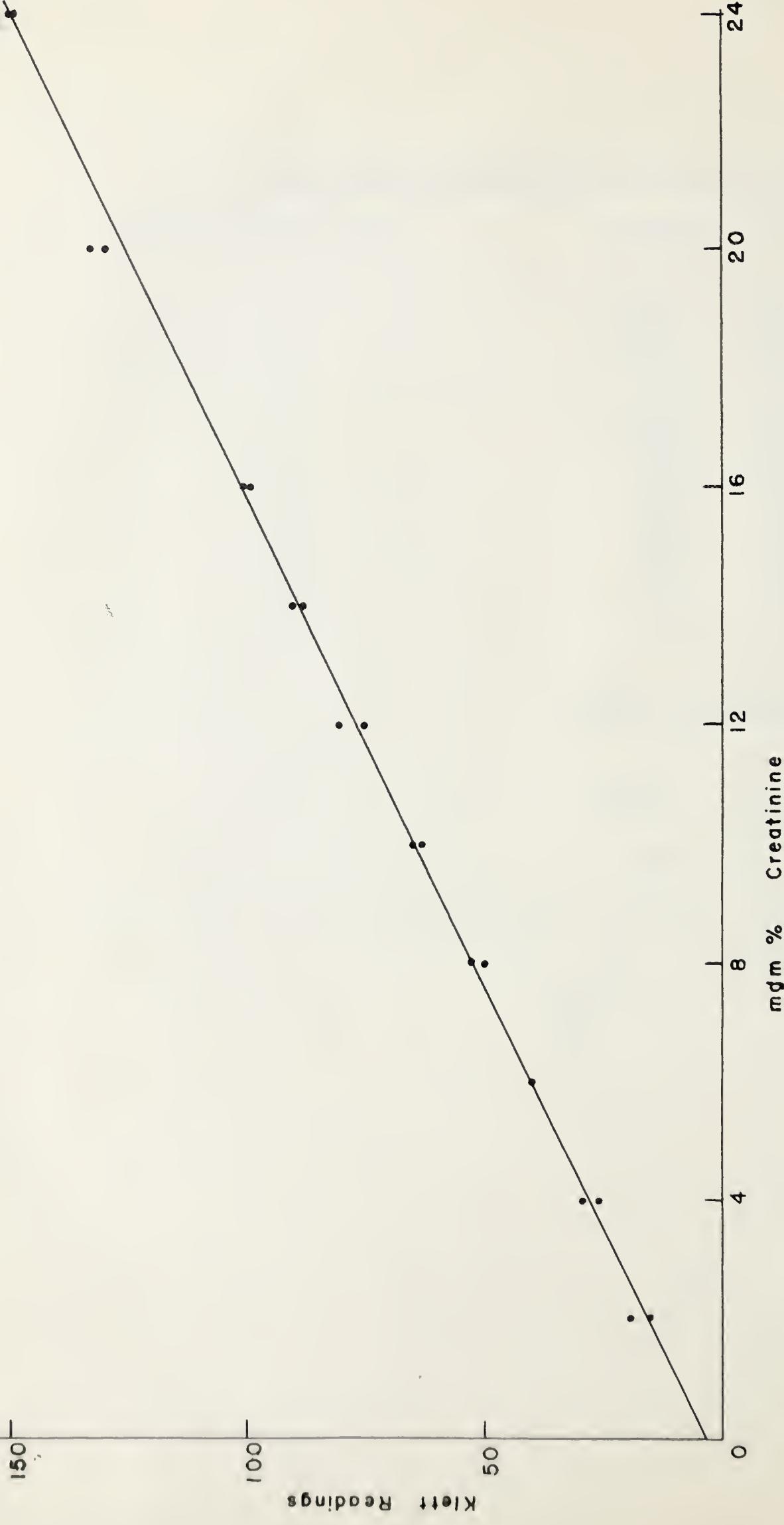
mgm % Creatinine

Raw Data for Plasma Creatinine Standard Curve (4/3/60)

Klett Readings		Mgm % Creatinine
0	0	0
15	19	2
26	29.5	4
40	40	6
52.5	50	8
65	63	10
75	80.5	12
90	88	14
99.5	100.5	16
130	133	20
149.5	150.5	24
172.5	169.5	28

Regression Values

$n = 24$

$byx = .1626$

$a = -0.46$

mgm % Creatinine $= K.R. \times .1626 - 0.46$

Plasma PAH Standard Curve
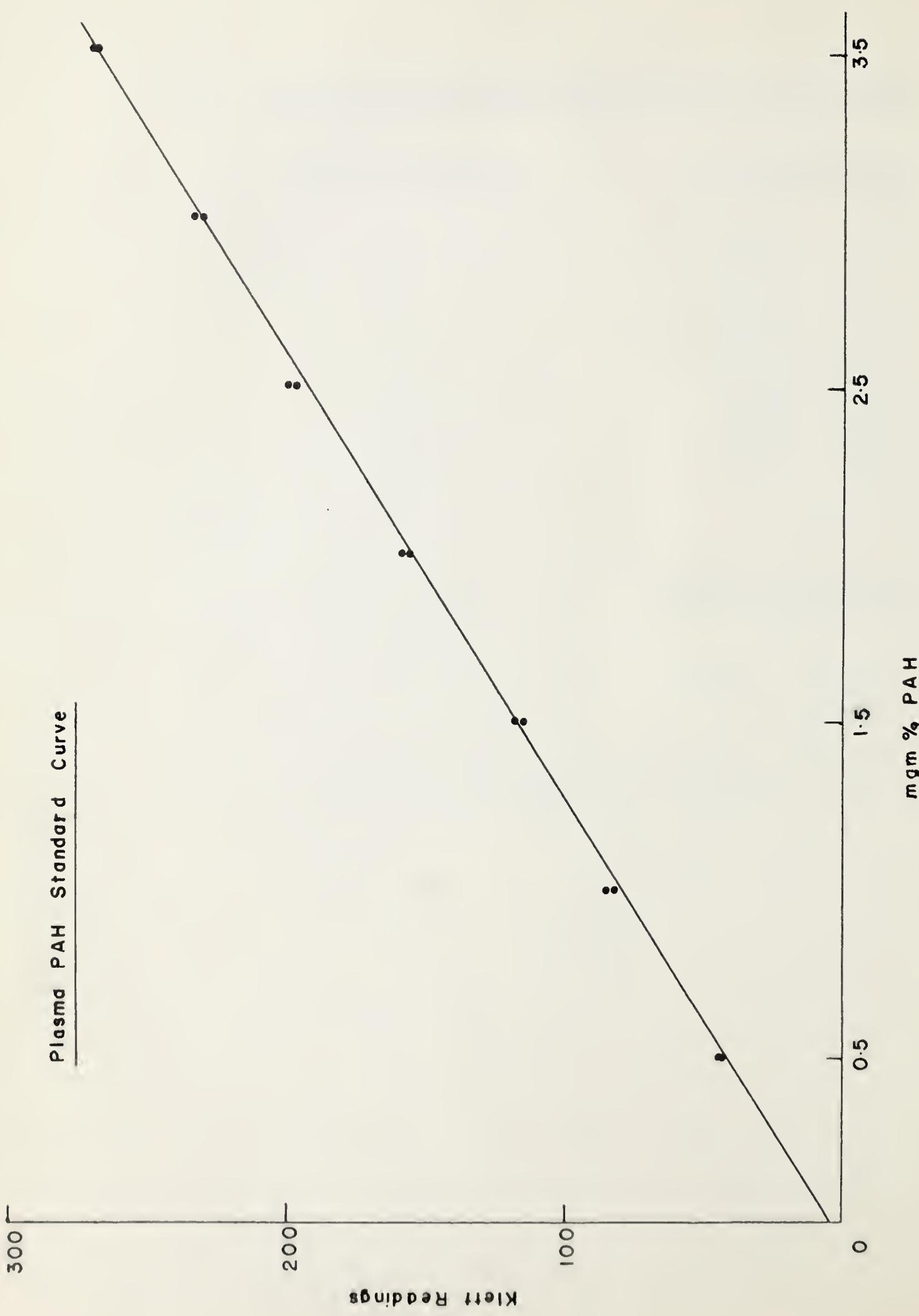
Raw Data for Plasma PAH Standard Curve (4/3/60)

Klett Readings		Mgm % PAH
0	0	0
43.5	44.5	0.5
85	82	1.0
115	118	1.5
156	158.5	2.0
197	200	2.5
234	231	3.0
271	269	3.5

Regression Values

$n = 16$

$byx = .01304$

$a = -0.05$

mgm % PAH $=$ K.R. x $0.01304 - .05$

Appendix IV

MOSMOLALITY STANDARD CURVES
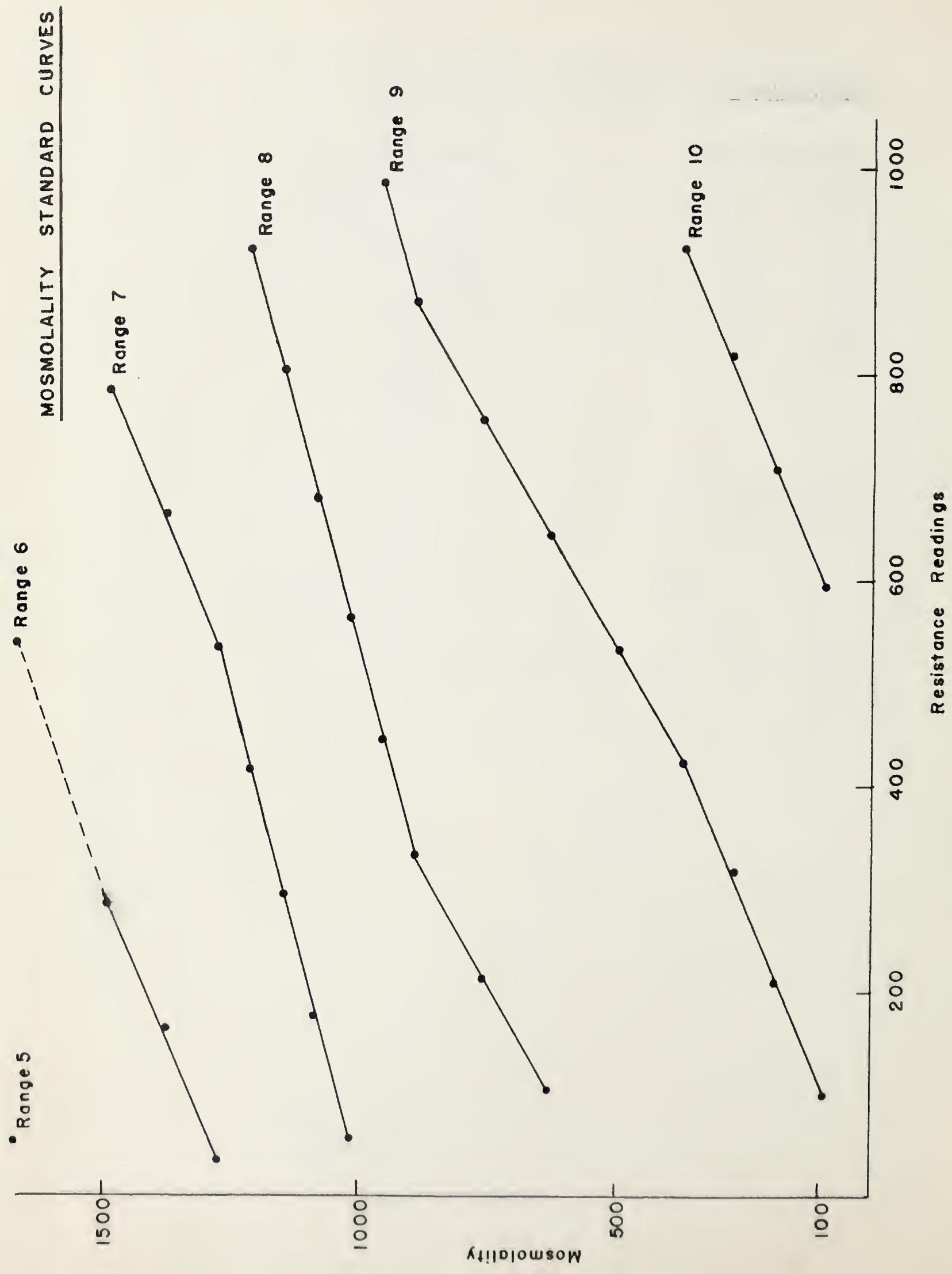
Mosmolality Raw Data

Mosm/Kg H$_2$O	Range	Resistance	Range	Resistance
94.5	10	596	9	98
187	10	707	9	208
277	10	817	9	315
368	10	922	9	421
497	9	532		
629	9	642	8	103
760	9	754	8	212
889	9	869	8	331
954	9	984	8	446
1019	8	563	7	56
1083	8	678	7	176
1145	8	802	7	295
1214	8	918	7	416
1275	7	534	6	34
1375	7	661	6	162
1484	7	781	6	288
1661	6	538	5	53
1830	6	836	5	328

Conversion Formula

$R_9 = R_{10} - 500$

$R_8 = R_9 - 539$

$R_7 = R_8 - 505$

$R_6 = R_7 - 497$

Regression was calculated for four different slopes at these intervals:

1. 0 - 368 mosm
    byx = 0.8414
    a_{10} = -408.3

    a_9 =   12.4

2. 368 - 889 mosm
    byx = 1.1648
    a_9 = -120.4

    a_8 =   507.4

3. 889 - 1275 mosm
    byx = 0.5453
    a_8 =   416.8

    a_7 =   710.7

    a_6 =   986.1

4. 1275 - 1484 mosm
    byx = 0.8338
    a_7 =   828.9

    a_6 = 1243.3

Range	Reading Interval	Formula
10	500-920	mosm = 0.8414X − 408.3
9	0-420	" = 0.8414X + 12.4
9	420-870	" = 1.1648X − 120.4
9	870-1000	" = 0.5453X + 416.8
8	0-330	" = 1.1648X + 507.4
8	330-1000	" = 0.5453X + 710.7
7	0-535	" = 0.5453X + 986.1
7	535-780	" = 0.8338X + 828.9
6	0-290	" = 0.8338X +1243.3

X = resistance reading

Appendix V

Raw Data for Effects of Meter Installation, Increased Venous Pressure and Anaesthetic on Renal Function.

Dog #	Date	Weight in Kg.
1	2/10/60	10.0
2	2/17/60	12.0
3	4/11/60	12.5
4	4/6/60	10.0
5	3/1/60	14.5
6	3/14/60	14.5
7	4/13/60	11.0
8	3/11/60	11.4
9	3/23/60	8.2
10	4/25/60	9.0

See Appendix I for guide to abbreviations.

CMPA Randomization		C				M			
Function	Side	1	2	3	4	1	2	3	4
ERPF	L	67.2	59.7	50.6	46.2	16.0	48.6	52.1	41.6
	R	49.6	59.9	63.2	42.5	18.0	49.4	59.3	37.2
ERBF	L	168	105	$\doteq\doteq$	95.3	34.1	86.6	106.7	83.7
	R	99.3	105	--	87.8	37.3	90.1	121.2	74.7
TRPF	L	--	--	--	57.2	18.1	62.2	76.5	48.8
TRBF	L	--	--	--	118	34.3	112.3	138	100
E$_{PAH}$	L	--	--	--	.812	.885	.771	.769	.847
MRBF	L	--	--	--	--	48.9	108	117	85
AP		165	150	110	128	126	137	130	122
RVP	L	9	10	9	11	19	26	27	30
GFR	L	16.2	39.7	18.4	17.3	4.2	25	17.4	14.7
	R	18.4	39.3	23.3	16.5	5.6	41.6	22.4	13.9
TFF	L	--	--	--	.30	.24	.40	.26	.30
V	L	1.8	2.4	7.0	3.0	1.3	3.8	8.7	2.6
	R	2.0	2.5	7.3	2.8	1.7	4.4	12.3	3.0
%WR	L	98.8	99.2	96.2	98.2	96.9	98.5	94.9	98.2
	R	98.8	99.3	96.9	98.3	96.9	99.0	95.5	97.8
Posm		323	323	314	326	350	336	322	342
Uosm	L	1172	1333	610	1110	930	1100	583	1096
	R	1103	1418	721	1140	887	1229	634	1016
UosmV	L	2060	3410	4250	3150	1240	4140	5040	2800
	R	2060	3480	5310	3040	1540	5370	7760	3030
%SR	L	95.8	97.0	92.6	94.3	91.7	95.0	90.9	94.4
	R	95.9	97.1	92.7	94.2	92.2	96.1	88.6	93.6
Cosm	L	6.4	10.5	13.4	9.6	3.5	12.3	15.3	8.3
	R	6.3	10.9	17.0	9.2	4.4	16.0	24.1	8.9

Function	Side	P				A		
		1	2	3	4	1	2	3
ERPF	L	14.4	37.2	46.5	32.5	32.7	34.3	42.7
	R	25.2	50.2	57.6	31.5	33.7	52.6	59.3
ERBF	L	30.5	67.2	89.2	59.8	69.3	64.2	79.2
	R	53.3	90.7	110.5	58	71.5	99.5	110
TRPF	L	16.4	45.2	60.9	37.7	39.3	45.3	55.4
TRBF	L	34.7	81.6	117	56.3	83.5	84.8	102.5
E_{PAH}	L	.878	.822	.763	.862	.815	.754	.771
MRBF	L	38.1	76.9	102	76.5	46.2	66.9	84
AP		124	141	134	131	103	127	130
RVP	L	34	33	34	33	29	28	29
GFR	L	3.4	19.7	15.1	13.1	5.9	20	13.1
	R	6.1	38.9	22	12.4	6.9	49.9	19.3
TFF	L	.21	.43	.25	.32	.15	.44	.24
V	L	1.3	2.6	7.8	3.3	2.0	2.8	6.7
	R	3.8	4.7	11.0	3.3	3.3	6.2	8.4
%WR	L	97.4	98.7	94.8	97.4	96.6	98.6	94.9
	R	95.8	98.8	95.0	97.3	95.3	98.8	95.7
Posm		355	338	333	348	355	345	343
Uosm	L	928	1182	513	1020	821	1130	581
	R	863	1224	613	995	792	1136	790
UosmV	L	840	2850	4000	3400	1660	3160	3870
	R	2180	5750	6700	3290	2410	7010	6630
%SR	L	93.0	95.3	92.1	92.5	92.1	95.5	91.4
	R	89.9	95.6	90.8	92.4	89.7	95.9	90.0
Cosm	L	2.35	9.2	12.0	9.8	4.68	9.4	11.3
	R	6.16	17.0	20.1	9.6	7.07	20.3	19.1

CMAP Randomization		C			M		
Function	Side	5	6	7	5	6	7
ERPF	L	67.8	37.3	69.9	32.8	23.3	59.5
	R	62.0	41.3	69.2	35.5	25.3	61.3
ERBF	L	122.7	85.2	143.8	59.2	53.0	113.8
	R	112.2	94.5	144.2	64.0	58.2	118
TRPF	L	85.3	--	--	41.2	29.3	68.5
TRBF	L	159	--	--	74.2	67.7	132.2
E_{PAH}	L	.795	--	--	.795	.797	.871
MRBF	L	--	--	--	65	57.9	125.5
AP		148	154	159	125	129	144
RVP	L	8	10	6	20	19	25
GFR	L	17.5	12.2	29.0	11.2	9.0	21.8
	R	15.7	13.5	30.5	12.0	9.3	28.0
TFF	L	.21	--	--	.27	.31	.32
V	L	4.8	4.5	7.2	4.2	4.3	6.7
	R	4.5	5.1	8.0	5.0	4.4	4.8
%WR	L	97.3	96.3	97.5	96.3	95.2	97.0
	R	97.2	96.2	97.4	95.9	95.3	98.1
Posm		342	333	325	349	350	333
Uosm	L	680	545	942	677	521	890
	R	656	589	928	673	567	1140
UosmV	L	3250	2480	6760	2820	2260	5930
	R	2950	3000	7380	3350	2500	5890
%SR	L	94.6	93.9	93.8	92.8	92.8	91.8
	R	94.5	93.3	93.5	92.0	92.4	93.6
Cosm	L	9.5	7.5	20.6	8.1	6.5	17.8
	R	8.3	9.0	22.7	9.6	7.2	16.7

Function	Side	A			P		
		5	6	7	5	6	7
ERPF	L	34.5	28.2	46.3	24.8	17.3	45.3
	R	38.8	30.2	57.3	36.5	28.5	66.0
ERBF	L	66.6	63	86.8	50.3	35.5	80.8
	R	75.5	67.3	107.2	73.8	58.0	117.3
TRPF	L	41.5	35.0	54.7	29.7	20.8	50.0
TRBF	L	80.7	78.2	102	60.0	42.5	90.0
E$_{PAH}$	L	.830	.804	.852	.852	.827	.835
MRBF	L	67	72.1	103.6	52	52.5	94.7
AP		114	100	138	106	101	135
RVP	L	21	23	21	28	26	32
GFR	L	12.3	10.6	18.5	9.7	5.9	17.0
	R	15.6	11.1	26.2	14.7	9.9	30.3
TFF	L	.30	.31	.21	.32	.28	.21
V	L	6.1	8.5	4.9	4.2	4.6	4.4
	R	8.2	9.4	4.8	7.5	10.4	4.2
%WR	L	95.0	91.9	97.4	95.6	92.2	97.4
	R	94.7	91.5	98.2	94.9	89.5	98.3
Posm		357	359	334	359	371	336
Uosm	L	704	428	978	781	439	999
	R	694	453	1241	821	402	1314
UosmV	L	4330	3650	4770	3290	2020	4420
	R	5720	4250	5910	6150	4080	6850
%SR	L	90.1	90.4	92.3	90.6	90.8	92.3
	R	89.7	89.3	93.2	88.3	89.1	93.2
Cosm	L	12.1	10.2	14.2	9.2	5.5	13.2
	R	16.0	11.7	17.6	17.1	11.3	20.4

CAMP Randomizations

Function	Side	C			A		
		8	9	10	8	9	10
ERPF	L	55.3	23.6	26.8	50.3	21.3	52.7
	R	67.5	29.7	66.2	53.8	23.6	45.2
ERBF	L	107.8	45.9	139	96.2	37.7	91.2
	R	131.2	57.8	131	103.2	41.6	80.6
TRPF	L	64.7	--	105.2	56.8	--	70.0
TRBF	L	125.8	--	208	108.2	--	125.3
E_{PAH}	L	.848	--	.661	.890	--	.727
MRBF	L	--	--	--	--	--	--
AP		166	129	145	162	152	145
RVP	L	7	12	7	5.5	10	6
GFR	L	21.6	9.4	18.9	20.6	10	12.5
	R	23.1	12.8	19.1	21.1	11.8	13.5
TFF	L	.35	--	.18	.36	--	.18
V	L	4.1	1.9	7.3	4.4	3.2	7.9
	R	3.6	3.4	6.7	3.6	4.5	7.2
%WR	L	98.1	97.9	96.1	97.8	96.2	93.7
	R	98.4	97.3	96.3	98.3	96.1	94.6
Posm		330	327	323	332	335	336
Uosm	L	950	922	460	1059	794	466
	R	973	790	493	1157	784	515
UosmV	L	3840	1780	3350	4700	2500	3680
	R	3470	2713	3290	4130	3470	3730
%SR	L	94.4	94.1	94.4	93.1	92.6	91.2
	R	95.3	93.5	94.4	94.1	91.0	92.0
Cosm	L	11.7	5.4	10.4	14.1	7.5	10.9
	R	10.5	8.3	10.2	12.4	10.3	11.1

Function	Side	M			P		
		8	9	10	8	9	10
ERPF	L	35.5	17.6	33.7	20.5	20.7	25.0
	R	33.0	23.5	44.8	20.2	25.6	32.7
ERBF	L	60.5	29.0	54.3	36.2	32.4	37.3
	R	59.8	38.6	72.5	35.5	40.0	49.0
TRPF	L	40.5	21.9	42.8	22.5	24.2	31.8
TRBF	L	73.5	35.9	68.8	39.8	37.9	47.6
E_{PAH}	L	.879	.807	.789	.904	.856	.785
MRBF	L	72.5	35.6	58.5	39.0	36.8	46.5
AP		155	156	112	156	150	108
RVP	L	18	--	14	25	--	17
GFR	L	16.4	8.2	9.8	9.6	8.7	6.4
	R	15.5	11.1	11.4	9.2	11.1	9.1
TFF	L	.40	.27	.23	.42	.28	.20
V	L	4.1	5.4	5.6	2.4	5.7	3.9
	R	2.8	6.7	6.8	1.8	6.8	5.9
%WR	L	97.5	93.4	94.3	97.4	93.5	93.9
	R	98.2	94.5	94.0	98.1	93.8	93.5
Posm		338	346	348	347	350	359
Uosm	L	1068	676	637	1053	731	599
	R	1069	737	586	1179	796	515
UosmV	L	4410	3670	3570	2540	4140	2340
	R	3310	4930	4010	2070	5470	3040
%SR	L	92.0	87.1	89.5	91.2	86.4	89.8
	R	93.7	87.5	89.8	93.5	85.9	90.7
Cosm	L	13.0	10.6	10.3	8.3	11.8	6.5
	R	9.6	14.2	11.5	6.0	15.6	8.4

L/R RATIOS

CMPA Randomization

		ERPF	GFR	Uosm	V	UosmV	Cosm
C	1	1.35	0.87	1.06	0.90	1.00	1.00
	2	1.00	1.01	0.94	0.96	0.97	0.96
	3	0.82	0.79	0.85	0.96	0.80	0.79
	4	1.09	1.05	0.98	1.07	1.04	1.03
M	1	0.89	0.75	1.05	0.76	0.81	0.81
	2	0.98	0.60	0.89	0.86	0.77	0.77
	3	0.87	0.78	0.92	0.71	0.65	0.64
	4	1.12	1.06	1.08	0.87	0.92	0.93
P	1	0.57	0.56	1.07	0.34	0.38	0.38
	2	0.74	0.51	0.97	0.55	0.50	0.54
	3	0.81	0.69	0.84	0.71	0.60	0.60
	4	1.03	1.05	1.03	1.00	1.03	1.02
A	1	0.97	0.86	1.04	0.61	0.69	0.66
	2	0.65	0.40	1.00	0.45	0.44	0.46
	3	0.72	0.68	0.74	0.80	0.58	0.59

CMAP Randomization

		ERPF	GFR	Uosm	V	UosmV	Cosm
C	5	1.09	1.11	1.04	1.07	1.10	1.14
	6	0.90	0.90	0.93	0.88	0.83	0.83
	7	1.01	0.95	1.01	0.90	0.92	0.91
M	5	0.92	0.93	1.00	0.84	0.84	0.84
	6	0.92	0.97	0.92	0.98	0.90	0.90
	7	0.97	0.78	0.78	1.40	1.01	1.07
A	5	0.89	0.79	1.01	0.74	0.76	0.76
	6	0.93	0.95	0.95	0.90	0.88	0.87
	7	0.81	0.71	0.79	1.02	0.81	0.81
P	5	0.67	0.66	0.95	0.56	0.53	0.54
	6	0.61	0.66	1.09	0.44	0.49	0.49
	7	0.69	0.56	0.76	0.85	0.65	0.65

CAMP Randomization

		ERPF	GFR	Uosm	V	UosmV	Cosm
C	8	0.82	0.94	0.98	·1.14	1.08	1.11
	9	0.79	0.73	1.15	0.56	0.66	0.65
	10	1.16	0.99	0.93	1.09	1.02	1.02
A	8	0.93	0.97	1.00	1.22	1.14	1.14
	9	0.90	0.85	1.01	0.71	0.72	0.73
	10	1.17	0.93	0.90	1.10	0.99	0.98
M	8	1.08	1.06	1.00	1.46	1.33	1.35
	9	0.75	0.74	0.92	0.81	0.74	0.75
	10	0.75	0.86	1.09	0.82	0.89	0.90
P	8	1.01	1.04	0.90	1.33	1.23	1.38
	9	0.81	0.78	0.92	0.84	0.73	0.76
	10	0.77	0.70	1.16	0.66	0.77	0.77